Mechanisms of Ageing and Development 132 (2011) 287–297

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Higher levels of heat shock proteins in longer-lived mammals and birds

Kurtis D. Salway, Emily J. Gallagher, Melissa M. Page, Jeffrey A. Stuart *

Department of Biological Sciences, Brock University, St. Catharines, ON, Canada L2S 3A1

ARTICLE INFO

Article history:
Received 9 November 2010
Received in revised form 3 June 2011
Accepted 6 June 2011
Available online 14 June 2011

Keywords:
Protein homeostasis
Maximum lifespan
Aging
Protein folding
Heat shock proteins

ABSTRACT

Cellular stress resistance is generally associated with longevity, but the mechanisms underlying this phenotype are not clear. In invertebrate models there is a clear role for heat shock proteins (Hsps) and organelle-specific unfolded protein responses (UPR) in longevity. However, this has not been demonstrated in vertebrates. Some Hsp amino acid sequences are highly conserved amongst mammals and birds. We used antibodies recognizing conserved regions of Hsp60 (primarily mitochondrial), Hsp70 (primarily cytosolic), GRP78 (Bip) and GRP94 (endoplasmic reticulum) to measure constitutive levels of these proteins in brain, heart and liver of 13 mammalian and avian species ranging in maximum lifespan from 3 to 30 years. In all three tissues, the expression of these proteins was highly correlated with MLSP, indicating higher basal levels of Hsp expression are characteristic of longer-lived species. We also quantified the levels of Hsp60, Hsp70 and GRP78 in brain and heart tissue of young adult (6–7 month old) Snell dwarf mice and normal littermates. Snell dwarf mice are characterized by a single gene mutation that is associated with an ~50% increase in lifespan. However, neither Hsp60, nor Hsp70, nor GRP78 levels were elevated in brain or heart tissue from Snell dwarf mice compared to normal littermates.

1. Introduction

Vertebrates are a diverse clade with a wide range of maximum lifespans (MLSPs) from several years to over a century. Yet, the phenotype of aging appears remarkably similar amongst these species, suggesting it may stem from common cellular and molecular mechanisms. Therefore, it may be possible to identify specific molecular traits that have co-evolved with longevity and indeed may facilitate it. We currently have a limited understanding of what these traits are and their determination remains a major goal. Previous studies have shown that the cells of longer-lived species are characterized by greater resistance to exogenous stressors (Kapahi et al., 1999; Kirkwood et al., 2000; Ogburn et al., 2001; Miller et al., 2011).

Protein homeostasis is a critical component of cellular maintenance and stress resistance. Oxidative stress causes damage that alters protein structure and function, and can promote aggregation, has been well characterized in several neurodegenerative diseases (Reviewed in Ali et al., 2010). The importance of maintaining proteins in their correct conformation is illustrated by the robust unfolded protein responses (UPR) that manifest in various cellular compartments, including cytosol, mitochondria and endoplasmic reticulum (ER). The core effectors of the UPR in any compartment are heat shock proteins (Hsps), which range in size and activity, but function in a broad variety of protein folding, refolding and chaperoning activities (reviewed in Bukau et al., 2006). The importance of Hsps in aging and longevity has been illustrated by experiments in *Caenorhabditis elegans* and *Drosophila* species. For example, overexpression of hsp-6 (Yokoyama et al., 2002) and hsp-16 (Walker and Lithgow, 2003) genes extend lifespan in *C. elegans*, and heat shock factor 1 (HSF-1), which transcriptionally regulates the heat shock response, is required for lifespan extension in daf-2 *C. elegans* mutants (Hsu et al., 2003). Overexpression of Hsps is also associated with lifespan extension in *D. melanogaster* (Morrow et al., 2010). In mice, however, the data are much more equivocal. Overexpression of Hsp70 and HSF1 have been associated with increased longevity in disease models (e.g. Gifondorwa et al., 2007; Steele et al., 2008; Hayashida et al., 2010). However, overexpression of Hsp70 in healthy mice does not extend lifespan (Vanhooren et al., 2008. Also, tissue Hsp mRNA levels are not globally upregulated in long-lived dwarf or growth hormone/insulin-like growth factor-1 signalling pathway deficient mice (reviewed in Swindell, 2009). Thus, there is a need to further investigate the relationship between tissue Hsp levels and longevity in mammals.

The cytosol, ER and mitochondria are distinct subcellular compartments within which protein folding states must be maintained independently. Hsp70 is one of the main cytoplasmic Hsps. In the ER, glucose-regulated protein 78 (GRP78) and 94 (GRP94) are key mediators of the UPR and thus major contributors to the ER stress response. Mitochondria also contain chaperones that support proper folding of newly translocated proteins and of those synthesized within the mitochondrial matrix. Hsp60 resides predominantly in the mitochondria, and has been shown to be

* Corresponding author. Tel.: +1 905 688 5550x4814; fax: +1 905 688 1855.
E-mail address: jstuart@brocku.ca (J.A. Stuart).

0047-6374/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mad.2011.06.002

selectively upregulated during the mitochondrial UPR (Corydon et al., 2005; Lee, 2005; Zhao et al., 2002).

In this study, we tested the hypothesis that the evolution of longevity in vertebrate endotherm species has selected for increasing protein folding capacities to promote protein homeostasis concomitantly with longer lifespans. We used antibodies raised to conserved epitopes to measure levels of mitochondrial Hsp60, ER GRP78 and GRP94, and cytosolic Hsp70 in liver, heart and brain of young adults representing 13 vertebrate endotherm species ranging in maximum lifespan (MLSP) from 3 to 30 years (Table 1). We used residual analysis to differentiate associations with body mass from those with MLSP, and Felsenstein's independent contrasts (Felsenstein, 1985) to address problems associated with non-independence of data points (i.e. species) due to shared evolutionary history.

Finally, we also measured Hsp60, Hsp70 and GRP78 in heart and brain from young adult Snell dwarf mice, a well-characterized intra-specific model of longevity, to examine whether the reported absence of uniform Hsp mRNA upregulation in long-lived dwarves (Swindell et al., 2009) is paralleled by protein levels.

2. Materials and methods

2.1. Materials

Chemicals were purchased from Bioshop (Burlington, ON, Canada) and Sigma–Aldrich (Oakville, ON, Canada; including Fluka and Caledon). BioRad protein dye was purchased from BioRad Laboratories (Hercules, CA, USA). Anti-Hsp60, anti-GRP94, anti-GRP78 and anti-Hsp70 primary antibodies were purchased from Abcam (Cambridge, Massachusetts, USA). Anti-rabbit and anti-goat secondary antibodies were purchased from Rockland Immunochemicals (Gilbertsville, Pennsylvania, US). Prestained broad range protein marker was obtained from BioLabs, (New England, Massachusetts, USA). Memcode reversible protein stain was purchased from Thermo Scientific (Rockford, IL, USA).

2.2. Animals

Between 3 and 6 individuals of each of 12 mammalian and 1 avian species were used in this study (Table 1) as in Salway et al. (2010). Species were selected on the bases of: (1) phylogenetic position; (2) MLSP; (3) routine diet and (4) availability. With respect to phylogenetic position, we confined our analysis to species with a similar overall body plan and physiology, i.e. vertebrate endotherms. Within this constraint, we sampled species within and between orders. We also attempted to maximize the range of MLSP represented by species included in the study. We also included species with exceptionally long lifespans for their body mass (zebra finch). The nature of the study meant that we were unable to directly control for differences in diet specific to a given species; however, our species collection includes graminovores, nucivores, insectivores, omnivores and carnivores and no trends were noted that related to dietary preference. We also included wild-caught species where possible to control for domestication effects. All animals were healthy young adults, and in most cases the exact age and sex of the animal were known.

2.3. Tissue sampling

Normal C57BL mice were euthanized by cervical dislocation following which brain, heart and liver tissues were immediately excised, flash frozen in liquid nitrogen and stored at −80 °C. Snell normal and dwarf mice were anaesthetized with isoflurane, injected with a mixture of 50 μg/g ketamine and 5 μg/g xylazine before tissue excision within 30–45 min post-mortem. These tissues were flash frozen in liquid nitrogen and stored at −80 °C. Norway rats were euthanized with sodium pentabarbitol sodium injection (Euthanyl®; 2 μg/g body wt.), and brain, heart, and liver tissues were removed and flash frozen in liquid nitrogen and stored at −80 °C. Tissues from 13-lined ground squirrels were collected at the University of Western Ontario (London, ON, Canada) as described in Page et al. (2009). Active (i.e. non-hibernating) animals were euthanized by Euthanyl overdose (270 mg/ml, 0.2 ml/100 g body wt.), brain, heart and liver tissue were rapidly removed, flash-frozen in liquid nitrogen, and stored at −80 °C. Zebra finch tissues were collected at Trent University (Peterborough, ON, Canada). Finch was euthanized with 2.0 mg/g body wt. sodium pentabarbitol (Euthasol®), followed by decapitation. Brain, heart and liver tissues were removed and flash frozen in liquid nitrogen and stored at −80 °C. Guinea pigs were euthanized, and heart, brain and liver tissues rinsed in 1× PBS and immediately frozen in liquid nitrogen and shipped to Brock University on dry ice. Similarly, excised tissues from euthanized dogs, hamsters and gerbils were immediately frozen in liquid nitrogen and shipped to Brock University on dry ice. Tissue from domesticated livestock was collected at a local abattoir during normal processing, with the exception of rabbits which were collected during processing from a local farmer. In all cases, brain, heart and liver tissues were collected from these animals within approximately 30 min post mortem. Tissues were frozen on-site in dry ice then brought to Brock where they were stored at −80 °C. Fresh white-tailed deer tissue (collected within 1 h post mortem) was obtained by hunters in Cobden (ON, Canada), following which brain, heart and liver tissue was collected and frozen at −20 °C for one month, and then transferred to Brock University where it was stored at −80 °C. Tissue sampling sites were unspecified for the white-tailed deer samples.

2.4. Preparation of tissue homogenates

Brain, heart and liver tissues were homogenized using a PowerGen 125 Homogenizer (Fisher Scientific, Ottawa, Ontario, Canada) in ice cold lysis buffer (50 mM HEPES, 20 mM KCl, 0.1 mM EDTA, 1 mM DTT, 5% glycerol and 0.1% NP40) at full speed for 3 cycles of 10 s with 10 s rest between each cycle. Following homogenization, samples were centrifuged at 10,000 × *g* for 20 min at 4 °C. Protein concentrations were determined using the Bradford technique with a BioRad protein assay kit and homogenates were aliquotted and stored at −80 °C.

Table 1
Sex, age, mass and maximal lifespan (MLSP) of the mammalian and avian species.

Common name	Scientific name	Number and sex	Age	Mass (kg)	MLSP (yrs)[a]	Source
Mammalia						
Snell mouse (DW/J)	*Mus musculus*	6 F (+/dw)	6–7 months	0.030	2.7	Jackson Laboratories (Bar Habor, ME, USA)
		6 F (dw/dw)	6–7 months	0.010	3.9	
C57BL/6Ncr1BR mouse	*Mus musculus*	4 F 4 M	4 months	0.028	3.5	Charles River (Wilmington, MA, USA)
Syrian hamster	*Mesocricetus auratus*	4; sex unknown	4–8 months	0.130	3.9	Equitech-Bio, Inc. (Kerrville, TX, USA)
Norway rat	*Rattus norvegicus*	6 M	3 months	0.550	5	Charles River (Wilmington, MA, USA)
Mongolian gerbil	*Meriones unguiculatus*	4; sex unknown	4–8 months	0.070	6.3	Equitech-Bio, Inc. (Kerrville, TX, USA)
13-lined ground squirrel	*Spermophilus tridecemlineatus*	8 F 3 M	Unknown	0.205	7	University of Manitoba Field Research Station (Carmen MB, Canada)
Rabbit	*Oryctolagus cuniculus*	4 F 1 M	3.5 months	2.60	11.8	Local abattoir (Fort Erie, ON, Canada)
Guinea pig	*Cavia porcellus*	4; sex unknown	Unknown	1.05	12	Rockland (Gilbertsville, PA, USA)
White-tailed deer	*Odocoileus virginianus*	3 M	2.5–3.5 years	110	21.6	Local hunters (Cobden, ON, Canada)
Domestic dog	*Canis familiaris*	3 M	2–8 years	11.0	24	Equitech-Bio, Inc. (Kerrville, TX, USA)
Yorkshire/Hampshire pig	*Sus scrofa*	2 F 3 M	6 months	99.9	27	Local abattoir (Fort Erie, ON, Canada)
Black angus/charlet cattle	*Bos taurus*	4 F 1 M	1.5–2.5 years	379.5	30	Local abattoir (Fort Erie, ON, Canada)
Sussex sheep	*Ovis aries*	5 F	6–12 months	29.5	19.6	Local abattoir (Fort Erie, ON, Canada)
Aves						
Zebra finch	*Taeniopygia guttata*	3 F 3 M	1 year	0.017	14.5	Trent University Animal Care Facility (Peterborough, ON, Canada)

[a] MLSP data from De Magalhaes et al. (2005).

2.5. Quantification of heat shock protein levels

Brain, heart and liver homogenates (20 μg) were separated by SDS-PAGE and electrotransferred to a PDVF membrane. Loading position was randomly varied to prevent biasing due to uneven transfer. In addition, even protein transfer was verified using Memcode reversible protein stain prior to immunoblotting and in rare instances where uneven transfer was detected membranes were destroyed. The membranes were then incubated in a blocking solution, consisting of 5% skim milk in 1× PBS, for 45 min at room temperature, and then probed with anti-Hsp60 (1:2500), anti-GRP78 (1:400), anti-Hsp70 (1:100) or anti-GRP94 (1:100 for liver; 1:250 for heart and brain) primary antibodies in 5% skim milk–1× PBS-T overnight at 4 °C. After several washes, membranes were then probed with anti-rabbit secondary antibody for 1 h at room temperature, then washed and visualized using an Odyssey infrared visual system (LI-COR Biosciences). Protein level quantification was performed using Odyssey Imaging Software, Version 1.0. Only eight of the total thirteen species could be run on a single protein gel, so we included an internal standard (a rat sample) in each gel (in a randomized lane) to normalize Hsp band intensity of each species to. We also employed a second method, normalizing Hsp

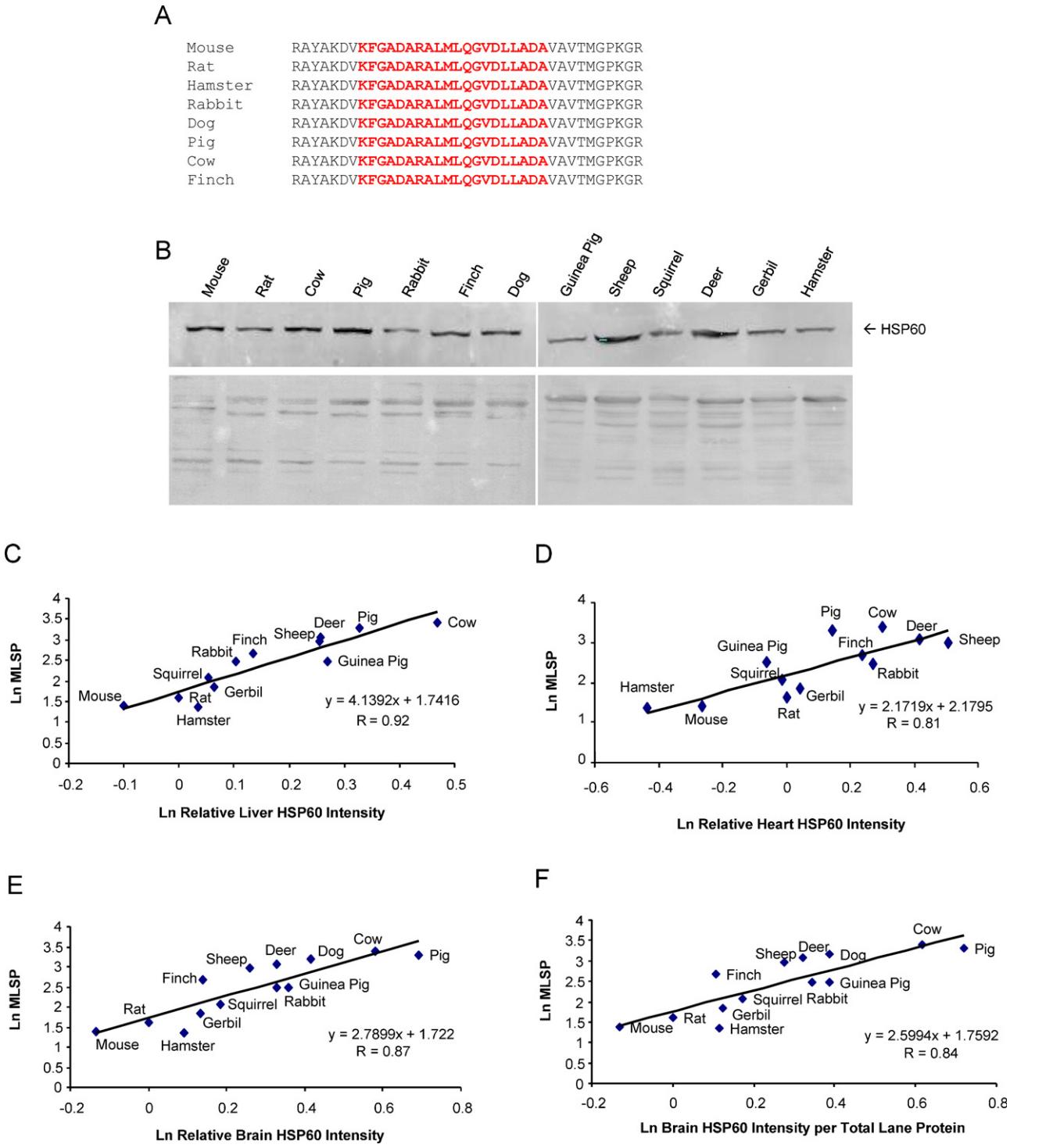

Fig. 1. Correlation between tissue Hsp60 levels and species MLSP. (A) Aligned HSP60 partial amino acid sequences showing region in which amino acid sequence identity is 100% and epitope (in red) to which antibody was raised. (B) Representative western blot of Hsp60, and corresponding Memcode stained membrane, in brain tissue of 13 species. Hsp60 protein levels were positively correlated ($p < 0.05$) with MLSP in (C) liver, (D) heart, and (E) brain. In (F) brain Hsp60 protein levels standardized to total lane Memcode signal are plotted against MLSP. This method of analysis produces essentially identical results. All values are natural-log transformed and each data point represents the mean of duplicate measurements made in 3–8 individuals of each species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

band intensities to the total Memcode stained protein signal in each lane. The two approaches gave virtually identical results (with respect to trends), and for subsequent analyses (calculation of residuals and FIC analysis) we used data normalized to the internal rat standard.

2.6. Statistical analyses

Raw data was natural logarithm (Ln)-transformed prior to correlation analyses. To remove the effects of body mass, residuals were calculated from simple linear regression of the dependent variable of interest on body mass. Significance of the coefficient of correlation was used to determine if the line was different from horizontal, and significance was based on t-values for one-tailed tests ($p < 0.05$ was considered significant). Felsenstein's phylogenetically independent contrasts (FIC; Felsenstein, 1985) were calculated using PDAP. The phylogenetic tree was constructed from four phylogenies from which branch length estimates were taken (Stuart et al., 2002; Springer and Murphy, 2007; Gorbunova et al., 2008; Hackett et al., 2008; Prasad et al., 2008); however, the analysis was repeated with all branch lengths set to one, as with a speciational model of character change (Price, 1997). Statistical analysis of Snell dwarf mouse data used a two-tailed independent t-test to compare normal mice and Snell dwarf mice with a p-value < 0.05 considered significant.

3. Results

Typically, quantitative immunoblotting of Hsp protein levels in tissues of different species requires identifying regions within the amino acid sequence of an individual Hsp that is 100% conserved amongst the species included in the study. Fortunately, the amino acid sequences of several Hsps are exceptionally highly conserved amongst vertebrates. However, while full genome sequence data are available for many of the species in Table 1, we were not able to

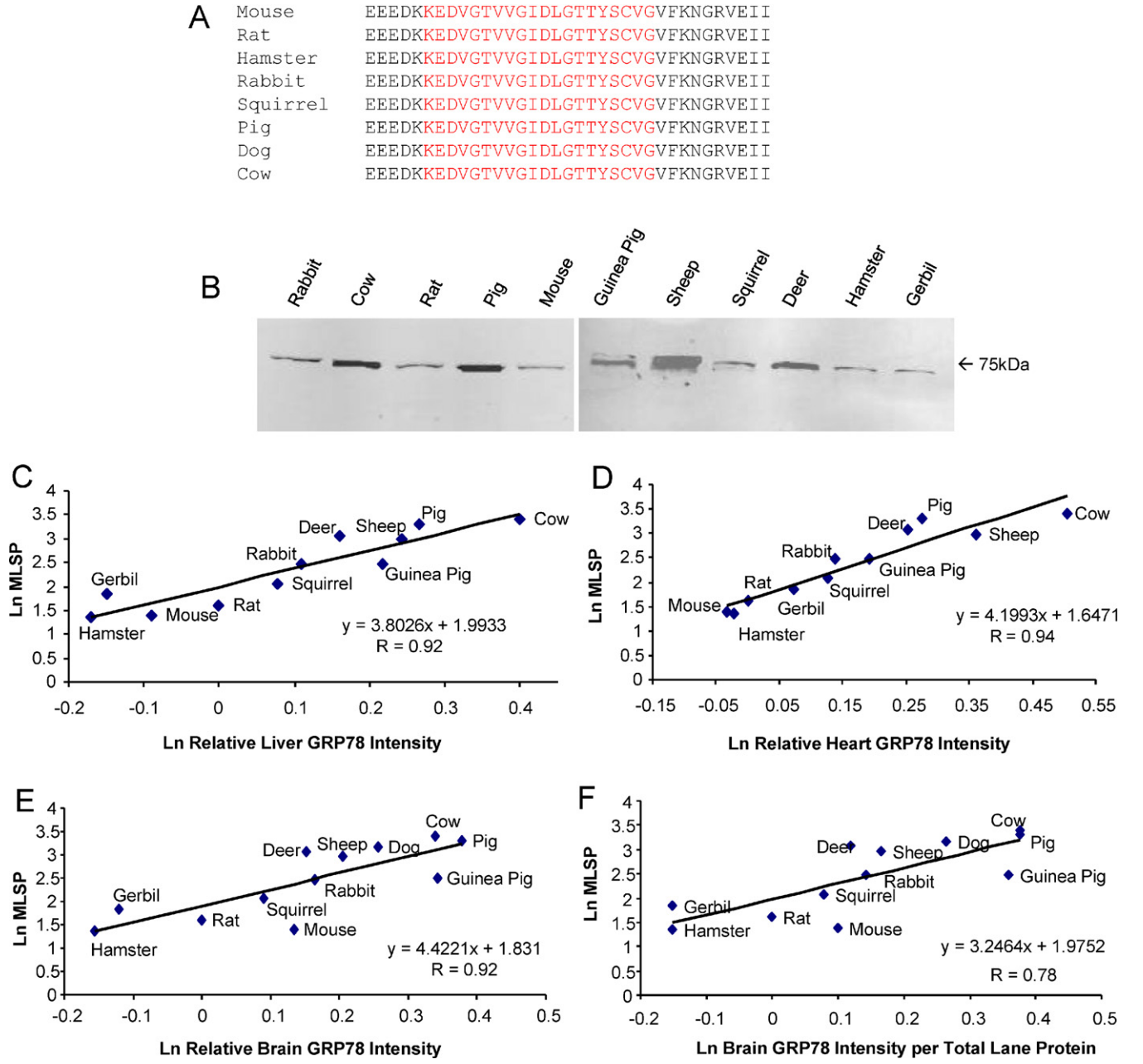

Fig. 2. Correlation between GRP78 levels and species MLSP. (A) Aligned GRP78 partial amino acid sequences showing region in which amino acid sequence identity is 100% and epitope (in red) to which antibody was raised. (B) Representative western blot of GRP78 in heart. GRP78 protein levels were positively correlated ($p < 0.05$) with MLSP in (C) liver, (D) heart, and (E) brain. In (F) brain GRP78 protein levels standardized to total lane Memcode signal are plotted against MLSP. This method of analysis produces essentially identical results. All values are natural-log transformed and each data point represents the mean of duplicate measurement from 3 to 8 individuals of a given species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

find sequence data for each Hsp for all species. For example, we found Hsp60 sequence data for eight species, including zebra finch (Fig. 1), and reasoned that a region 100% conserved across these relatively phylogenetically divergent species was likely to be conserved also in the other five. A similar logic was applied when identifying suitable regions for GRP78 and GRP94, with available amino acid sequences and alignments across species shown in Figs. 2 and 3. In each case, a commercial antibody was found that had been raised to an epitope within the region shown to be 100% conserved in all species for which sequence data was available. In the case of GRP78 (also called Hsp70-5 or Bip), it was necessary, in addition to the above condition, to select a sequence falling outside of regions that are highly conserved amongst different Hsp70 isoforms (see Daugaard et al., 2007). We selected a region near the N-terminus of GRP78 (Fig. 2) that, while fully conserved across nine species for which sequence data could be found, is substantially divergent between Hsp70 isoforms. The one exception to this approach was for Hsp70. In this instance, we did not find an antibody to a region that was fully conserved amongst the species included in this study while also being substantially divergent between Hsp70 isoforms. Instead, we probed a region near the C-terminus of Hsp70 using an antibody that was designed to detect primarily Hsp70-1a and Hsp70-1b but may also bind to Hsp70-2, Hsp70-6 or Hsc, which have up to 70% sequence identity in this region. Therefore, interpretation of our 'Hsp70' measurements must take this into account. For each Hsp, an alignment of available sequences and the epitope to which the antibody used had been raised are indicated (Figs. 1–4).

Hsp60 levels in liver, heart and brain were determined in 12–13 species (not all tissues were available for each species). In each case, band intensity was normalized to the rat value for that particular tissue (Table 2). A correlational analysis revealed highly significant positive correlations ($p < 0.05$) in liver, heart and brain tissues between MLSP and Hsp60 protein levels (Fig. 1B–D). In

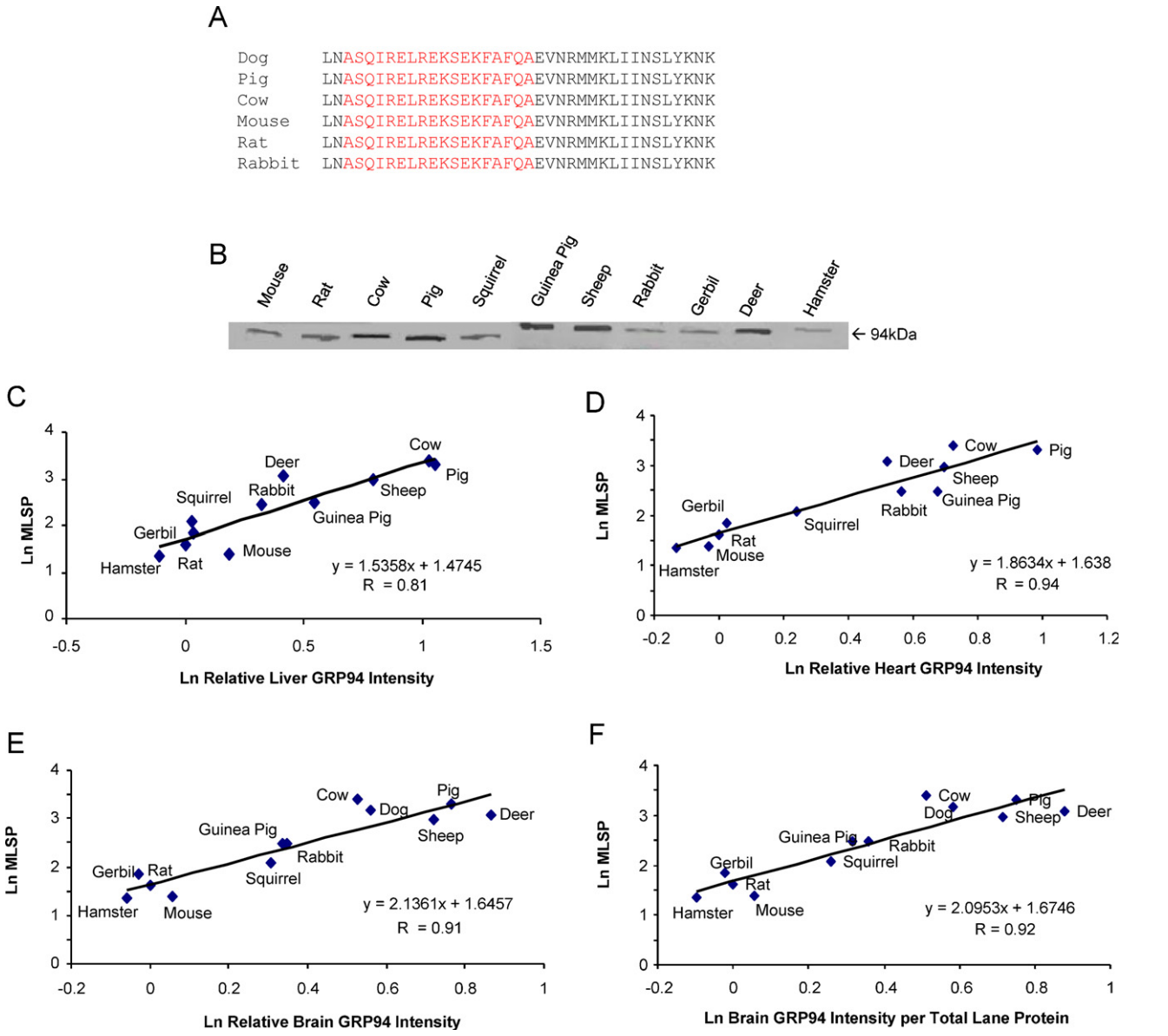

Fig. 3. Correlation between GRP94 levels and species MLSP. (A) Aligned GRP94 partial amino acid sequences showing region in which amino acid sequence identity is 100% and epitope (in red) to which antibody was raised. (B) Representative western blot of GRP94 in liver. GRP94 protein levels are positively correlated ($p < 0.05$) with MLSP in (C) liver, (D) heart and (E) brain. In (F) Brain GRP94 protein levels standardized to total lane Memcode signal are plotted against MLSP. This method of analysis produces essentially identical results. All values are natural-log transformed and each data point represents the mean of duplicate measurement from 3 to 8 individuals of a given species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

vertebrate endotherms, MLSP is highly correlated with body mass, and some relationships that appear to be with MLSP are actually driven by body mass. Therefore, to test whether a given correlation was actually driven by body mass, we plotted the residuals of the body mass versus Hsp60 protein levels against the residuals of body mass and MLSP. This analyses also yielded significant positive correlations ($p < 0.05$) between MLSP and Hsp60 levels for each tissue (Table 3). As the data points, representing species, are not independent owing to their shared phylogenetic history, a FIC analysis was also performed to account for this limitation. The positive correlations between Hsp60 levels and MLSP remained significant in each case ($p < 0.05$; Table 3). In addition, to control for the possibility that unequal loading/transfer of sample proteins influenced the observed trends, we repeated the analyses standardizing Hsp signals to the total Memcode stained protein signal in each lane. This analysis yielded essentially identical trends for Hsp60 (Fig. 1F), GRP78 (Fig. 2F), GRP94 (Fig. 3F) and Hsp70 (Fig. 4F) for each tissue (only brain tissue results shown).

The levels of two ER Hsps, GRP78 and GRP94, were measured using the same basic approach. GRP78 was strongly correlated ($p < 0.05$) with MLSP in all three tissues (Fig. 2). Analysis of residuals and application of FIC, as above, showed significant positive correlations ($p < 0.05$) of GRP78 with MLSP remained for each tissue. Identical results were obtained for GRP94, which was correlated with MLSP in liver and heart ($p < 0.05$; Fig. 3; Table 3), but the correlation in brain was significantly weakened ($p < 0.1$) following analysis of residuals and correction using FIC.

Hsp70 levels were quantified using the same approach as above. Similarly, correlational analysis revealed significant positive correlations ($p < 0.05$) in each of the three tissues between MLSP and Hsp70 protein levels (Fig. 4). These correlations remained following residual analysis and FIC analysis ($p < 0.05$; Table 3).

Hsp expression is affected by a variety of variables, including age (basal Hsp levels and/or inducibility may be reduced in individuals of advanced age). Although we selected animals that

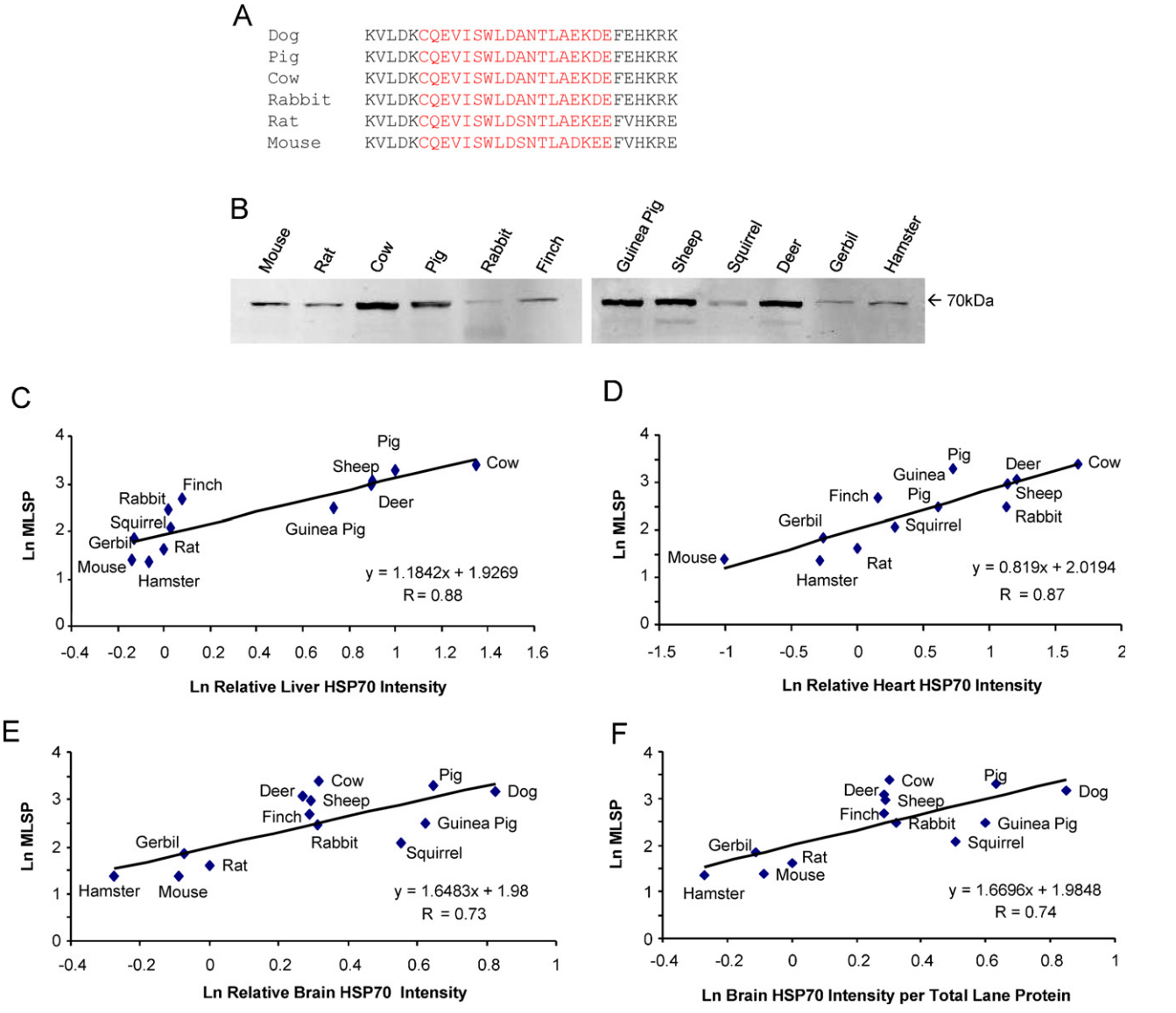

Fig. 4. Correlation between tissue Hsp70 levels and MLSP. (A) Aligned HSP70 partial amino acid sequences showing region in which amino acid sequence identity is highly conserved, and the epitope (in red) to which antibody was raised. (B) Representative western blot of Hsp70 in heart. Hsp70 protein levels were positively correlated ($p < 0.05$) with MLSP in (C) liver, (D) heart and (E) brain. In (F) brain Hsp70 protein levels standardized to total lane Memcode signal are plotted against MLSP. This method of analysis produces essentially identical results. All values are natural-log transformed and each data point represents the mean of duplicate measurement from 3 to 8 individuals of a given species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Heat shock protein expression relative to rat for each species from three different tissues.

Species/tissue	HSP60	HSP70	GRP78	GRP94
Liver				
Mouse	0.905 ± 0.133	0.869 ± 0.214	0.914 ± 0.045	1.205 ± 0.070
Hamster	1.036 ± 0.327	0.878 ± 0.053	0.843 ± 0.092	0.898 ± 0.051
Rat	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Gerbil	1.066 ± 0.291	0.936 ± 0.062	0.861 ± 0.071	1.039 ± 0.044
Squirrel	1.055 ± 0.423	1.028 ± 0.084	1.081 ± 0.089	1.027 ± 0.045
Rabbit	1.109 ± 0.158	1.018 ± 0.097	1.115 ± 0.023	1.379 ± 0.264
Guinea pig	1.308 ± 0.157	2.084 ± 0.338	1.244 ± 0.071	1.724 ± 0.085
Finch	1.144 ± 0.129	1.080 ± 0.193	n.d.	n.d.
Sheep	1.290 ± 0.430	2.444 ± 0.357	1.274 ± 0.074	2.209 ± 0.413
Deer	1.293 ± 0.264	2.455 ± 0.502	1.174 ± 0.049	1.519 ± 0.020
Pig	1.389 ± 0.096	2.709 ± 0.423	1.303 ± 0.098	2.867 ± 0.54
Cow	1.598 ± 0.251	3.846 ± 0.571	1.490 ± 0.109	2.80 ± 0.249
Heart				
Mouse	0.768 ± 0.252	0.364 ± 0.030	0.986 ± 0.169	0.969 ± 0.083
Hamster	0.647 ± 0.133	0.775 ± 0.086	1.026 ± 0.056	0.875 ± 0.044
Rat	1 ± ± 0	1 ± 0	1 ± 0	1 ± 0
Gerbil	1.045 ± 0.138	0.755 ± 0.173	1.075 ± 0.096	1.023 ± 0.188
Squirrel	0.989 ± 0.027	1.335 ± 0.178	1.154 ± 0.088	1.273 ± 0.039
Rabbit	1.310 ± 0.330	3.106 ± 0.789	1.147 ± 0.143	1.757 ± 0.185
Guinea pig	0.939 ± 0.121	1.840 ± 0.369	1.211 ± 0.112	1.967 ± 0.26
Finch	1.269 ± 0.401	1.166 ± 0.137	n.d.	n.d.
Sheep	1.657 ± 0.351	3.116 ± 0.610	1.434 ± 0.244	2.005 ± 0.116
Deer	1.517 ± 0.439	3.332 ± 0.093	1.289 ± 0.115	1.682 ± 0.13
Pig	1.156 ± 0.201	2.059 ± 0.474	1.336 ± 0.045	2.68 ± 0.436
Cow	1.349 ± 0.183	5.345 ± 0.696	1.654 ± 0.076	2.06 ± 0.076
Brain				
Mouse	0.875 ± 0.244	0.874 ± 0.106	1.144 ± 0.462	1.058 ± 0.114
Hamster	1.094 ± 0.085	0.759 ± 0.146	0.856 ± 0.181	0.944 ± 0.028
Rat	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Gerbil	1.143 ± 0.067	0.930 ± 0.108	0.886 ± 0.100	0.973 ± 0.006
Squirrel	1.204 ± 0.063	1.739 ± 0.316	1.093 ± 0.128	1.357 ± 0.123
Rabbit	1.311 ± 0.214	1.262 ± 0.158	1.178 ± 0.147	1.416 ± 0.184
Guinea pig	1.433 ± 0.146	1.861 ± 0.274	1.409 ± 0.219	1.401 ± 0.11
Finch	1.149 ± 0.136	1.231 ± 0.165	n.d.	n.d.
Sheep	1.300 ± 0.056	1.338 ± 0.398	1.228 ± 0.080	2.053 ± 0.202
Deer	1.389 ± 0.035	1.307 ± 0.064	1.164 ± 0.069	2.375 ± 0.370
Dog	1.514 ± 0.140	2.284 ± 0.417	1.293 ± 0.158	1.934 ± 0.569
Pig	1.997 ± 0.088	1.906 ± 0.294	1.460 ± 0.255	2.145 ± 0.342
Cow	1.789 ± 0.201	1.372 ± 0.108	1.405 ± 0.269	1.692 ± 0.216

n.d. = not determined.

were in the first three of their MLSP, it was considered possible that age at death might be a confounding factor in terms of Hsp expression levels. We therefore determined whether there was a relationship between the age of individuals at the time of euthanization and tissue Hsp levels. For this analysis, guinea pig and 13-lined ground squirrel were omitted because we had insufficient information regarding their exact age. There were no significant correlations between animal age (as a proportion of MLSP) and the level of any Hsp in any tissue (Table 4; Fig. 6 shows results of this analysis for brain tissue). Although the correlation between Hsp70 and animal age was near-significant ($p = 0.062$) for Hsp70 in brain (Fig. 4D), this result was unduly influenced by a single data point (dog). Omission of the dog value (Fig. 4E) renders the correlation highly non-significant ($p = 0.300$), and we thus conclude that there is no significant relationship between age at death and Hsp levels in the individual animals included in this study, presumably because all of these individuals were young adults.

IGF-1 signalling may also be an important pathway regulating the expression of Hsps, and plasma IGF-1 levels are negatively correlated with body mass in mammals (Stuart and Page, 2010). We therefore analyzed the relationship between plasma IGF-1 and tissue Hsp levels using IGF-1 data from Stuart and Page (2010), but again found no correlations between published plasma IGF-1 levels and Hsp expression in any tissue for the species included in the present study (data not shown).

We measured Hsp60, Hsp70 and GRP78 in heart and brain tissue from young adult Snell normal and dwarf mice, an intra-specific model of extended longevity. No significant differences between genotypes in Hsp70 or GRP78 protein levels were found in heart tissue, but Hsp60 was significantly lower in Snell dwarf mice compared to normal littermates ($p < 0.05$; Fig. 5). In brain tissue, there were no significant differences in the levels of any of the three Hsps between Snell normal and dwarf mice (Fig. 5).

4. Discussion

Our data indicate that elevated Hsp expression in heart, brain and liver tissues are characteristic of longer-lived species. Combined with published studies showing the cause and effect relationship between Hsp expression and longevity in invertebrates, this suggests that Hsps are pro-longevity across metazoan phyla. While we measured only a small representative sample of Hsps, which number in the dozens in vertebrates (Chen et al., 2006; Daugaard et al., 2007; Calderwood et al., 2009), it is possible that other Hsps are similarly correlated with lifespan.

There is surprisingly little information regarding the effects on lifespan of Hsp overexpression in mammals, with reported data largely confined to experiments in which Hsp overexpression has successfully rescued premature aging and death in murine models of disease (e.g. McArdle et al., 2004; McLean et al., 2002). However,

Table 3
Statistical analysis of linear regressions: heat shock protein expression as a function of MLSP.

Protein level	Correlation (R^2)	Slope	p-value
Liver HSP60			
Residual	0.5668	3.7098	0.002
Residual + FIC	0.4644	2.6636	0.011
Heart HSP60			
Residual	0.4583	1.3768	0.008
Residual + FIC	0.3725	1.0472	0.023
Brain HSP60			
Residual	0.3795	2.0464	0.013
Residual + FIC	0.3598	1.496	0.022
Liver HSP70			
Residual	0.3697	1.0826	0.020
Residual + FIC	0.3516	0.995	0.027
Heart HSP70			
Residual	0.3529	0.6493	0.021
Residual + FIC	0.3174	0.8171	0.036
Brain HSP70			
Residual	0.3772	0.6607	0.013
Residual + FIC	0.3887	0.7246	0.015
Liver GRP78			
Residual	0.3601	1.656	0.026
Residual + FIC	0.3433	2.1436	0.038
Heart GRP78			
Residual	0.4129	2.0904	0.017
Residual + FIC	0.6394	3.149	0.003
Brain GRP78			
Residual	0.3307	1.2457	0.042
Residual + FIC	0.3300	0.9346	0.040
Liver GRP94			
Residual	0.3315	0.6436	0.032
Residual + FIC	0.4261	0.7512	0.020
Heart GRP94			
Residual	0.6744	0.9672	0.001
Residual + FIC	0.695	1.3334	0.001
Brain GRP94			
Residual	0.3001	0.9677	0.053
Residual + FIC	0.3058	1.058	0.051

Table 4
Statistical analyses of linear regressions: heat shock protein expression as a function of animal age at death (as a fraction of MLSP).

Protein level	Correlation (R^2)	Slope	p-value
Hsp60			
Liver Hsp60	0.006	0.0135	0.415
Heart Hsp60	0.002	0.4554	0.450
Brain Hsp60	0.017	0.0466	0.350
GRP78			
Liver GRP78	0.002	0.0073	0.452
Heart GRP78	0.013	0.0203	0.377
Brain GRP78	0.043	0.0774	0.283
GRP94			
Liver GRP94	0.005	−0.0049	0.429
Heart GRP94	0.036	−0.0144	0.312
Brain GRP94	0.101	0.0633	0.183
Hsp70			
Liver Hsp70	0.043	0.0107	0.283
Heart Hsp70	0.001	0.0013	0.460
Brain Hsp70	0.168	0.0967	0.062

Vanhooren et al. (2008) showed that transgenic overexpression of Hsp70 in mice actually shortened lifespan, while increasing tumour incidence. Thus, global Hsp70 overexpression, perhaps via its anti-apoptotic capacity in highly mitotic cancer cells, may negate the value of its protective function by promoting tumorigenesis. It would be interesting to know which cell types contribute to the elevated Hsp levels in tissues measured in the longer-lived species studied here. It seems reasonable that Hsp levels might be differentially expressed in different cell types. The abundant highly oxidative and highly differentiated cell types that comprise tissues like brain and heart (e.g. myocytes, neurons) rather than actively mitotic cells are likely to be the primary contributors to the signals measured using our approach. However, a quantitative immunohistochemistry approach might provide insight into whether patterns of expression are unique to different cell types.

In addition to the potential for tumorigenesis, the transgenic manipulation of Hsp expression in mice may be complicated by the fact that these proteins play a variety of critical roles in development that could be negatively affected by lifelong systemic over- or under-expression. This is also evident in Hsp70 overexpressing mice (Vanhooren et al. 2008), in which reduced body mass is evident as early as one month of age. The approach we have taken circumvents these kinds of problems, as normal constitutive expression levels are not disrupted. Nonetheless, this comparative approach is necessarily limited by inherent inaccuracies in the estimation of MLSP for individual species. These values are highly biased by the number of observations that can be made for each species: in most species MLSP estimates tend to be revised upward with increasing numbers of observations. Here we limited the negative effects of this issue by utilizing a relatively large collection of 13 species.

The positive correlation of Hsp levels with MLSP is interesting in light of our previous findings, using the same collection of vertebrate species, that other components of the protein homeostasis machinery show no correlation with lifespan (Salway et al., 2010). Levels of protein ubiquitylation do not show any relationship with species MLSP. Similarly, the activities of the 20S/26S proteasome and protein redoxins capable of reversing oxidative protein damage do not correlate with MLSP (Salway et al., 2010). Page et al. (2010) found no evidence that antioxidant enzyme capacity is globally enhanced in long-lived species (Page et al., 2010). Thus, the positive correlation of protein folding and chaperoning activities with MLSP appears to be somewhat unique.

Our results could shed light on those of Perez et al. (2009) and Salmon et al. (2009) who showed that proteins from longer-lived species are resistant to unfolding in an in vitro assay. Although Perez et al. indicated that Hsp70 and Hsc70 levels in liver were not different between short-lived mice and the long-lived naked mole rat, these data were not shown nor was information regarding the sequences of the naked mole rat proteins, which would be required to confidently conclude this. Therefore, it remains possible that higher levels of Hsp70, or of other Hsps not measured, might contribute to the resistance to protein unfolding observed in these studies. This seems intuitively more plausible than evolving enhanced structural stability via amino acid sequence changes in the entire protein complement. From an energetic standpoint, the evolution of greater Hsp levels to facilitate protein chaperoning, refolding or disaggregation might also be more favourable than enhancing the capacity to recycle damaged proteins via enhanced proteasomal activities, which would incur the costs of both degradation and re-synthesis. Both Perez et al. (2009) and Salmon et al. (2009) similarly demonstrated that 20S/26S proteasome activity in liver is not elevated in young adult naked mole rats or bats compared to mice. Taken together, the evidence suggests a broad evolutionary pattern of increased Hsp expression, rather than protein degradation, oxidative damage repair, or amino acid substitution (shown by Moosmann and Behl, 2008; Bender et al., 2008 for mtDNA encoded, but not nuclear encoded proteins) to improve protein homeostasis in longer-lived species. Of course, this does not preclude the evolution of novel strategies within particular species that may diverge from this general pattern.

All of the Hsps measured here are expressed both constitutively and inducibly. Therefore, it is possible that elevated levels of Hsp

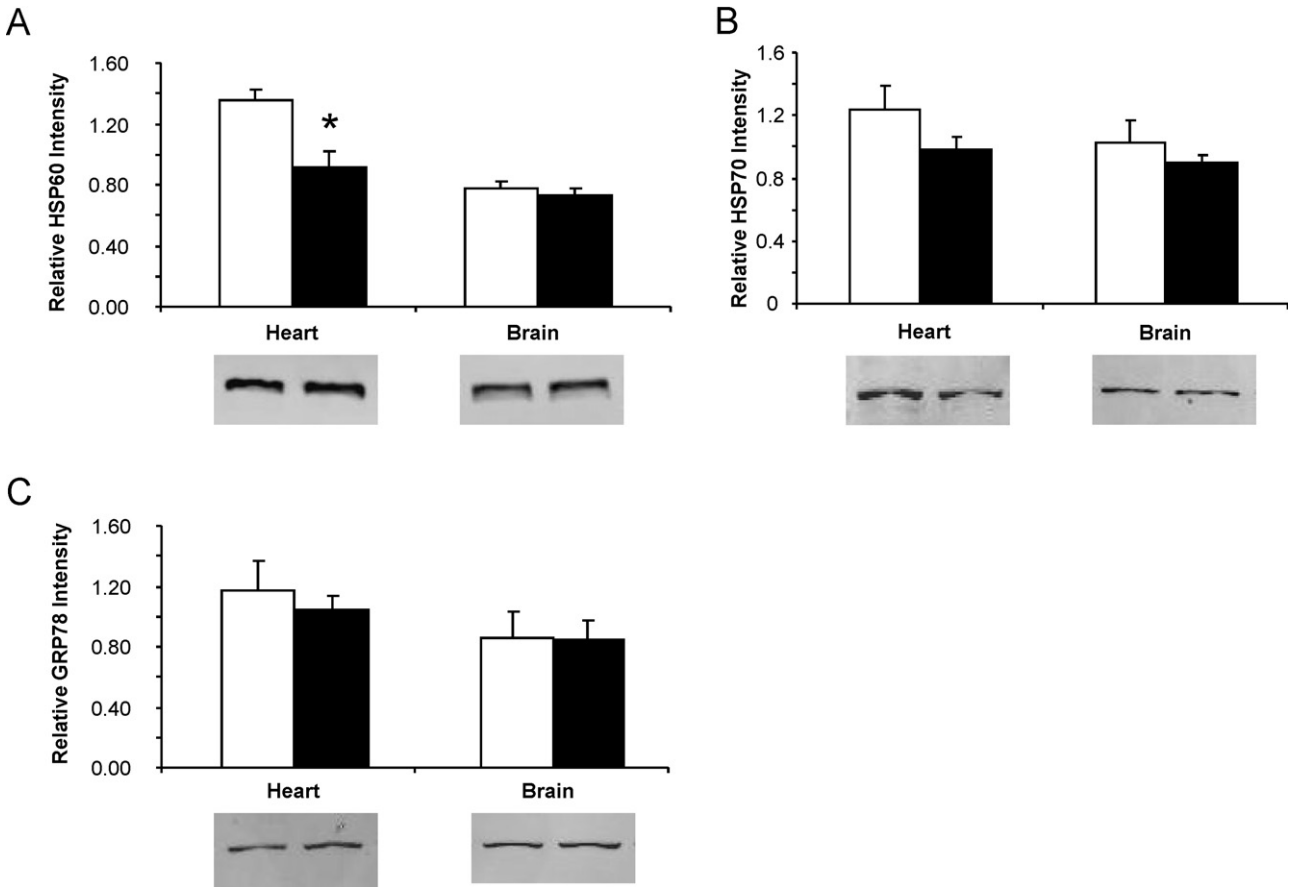

Fig. 5. Heat shock proteins are not upregulated in long-lived Snell dwarf mice compared to normal littermates. Relative (A) Hsp60; (B) Hsp70; (C) GRP78 protein levels and representative western blots in heart and brain tissue of young adult Snell dwarf mice and normal littermates. White bars represent Snell normal mice and black bars represent Snell dwarf mice. Values are means ± SEM of duplicate measurements made on tissue samples from each of five individuals per genotype. '*' = significantly different from normal mice ($p < 0.05$) using a two-tailed *t*-test.

expression in longer-lived species is a result of higher cellular stress under basal conditions. Alternatively, it is possible that these individuals experienced more stress during the process of euthanization and tissue excision. However, with the exception of deer which were taken by local hunters, conditions surrounding euthanization were strictly controlled for all animals used in this study. Perhaps most importantly, none of the animals were unduly stressed prior to euthanization and the time to tissue excision and freezing was similar in all circumstances. In any case, were such differences to be important they would most likely have increased variability of the data rather than introduced an artefactual trend, and correlation coefficients of the raw data were exceptionally high in most instances. Thus, the observed trends appear to reflect real differences in basal levels of Hsp expression associated with longevity that would facilitate improved protein homeostasis under steady state conditions. Although it is interesting to ask whether the longer-lived species from this study would also be better able to upregulate Hsp expression in response to an imposed stress, it was not practical to pursue this line of investigation in this study. This can, however, be investigated in cells that can be maintained and manipulated in culture. We are currently studying skeletal myoblasts collected from a subset of the species included here and finding similar results.

Although not all correlations of brain Hsp levels with MLSP were statistically significant, there was nonetheless a general pattern of higher Hsp levels in brain tissue from longer-lived species. This may provide an important insight into how longer-lived animals maintain normal brain function over their lifespans. Although we did not include human tissue in this study (because we could not adequately control time to tissue sampling), these results are likely to be relevant to humans. Neurodegenerative diseases are generally classified as protein misfolding diseases, often characterized by accumulation of abnormal protein aggregates. Overexpression of Hsps has generally been associated with attenuation of disease severity, while reduced function increases severity in a variety of models of mammalian neurodegeneration (McLean et al., 2002; Dou et al., 2003). While Hsp70 has been perhaps most studied in this context (Yoo et al., 1999; Dedmon et al., 2005), available evidence suggests similar roles for Hsp60 (e.g. Magen et al., 2008), Grp78 (Kudo et al., 2008), and Grp94 (Dukes et al., 2008). Thus, the co-evolution of increased basal Hsp expression with longevity suggests improved protein homeostasis in brain tissue and resistance to neurodegenerative disease in longer-lived species.

Although Hsp levels were generally correlated with species MLSP, there was no evidence that they were expressed at constitutively higher levels in long-lived Snell dwarf mice relative to normal littermates. This is consistent with the recent demonstration that Hsp mRNA levels in various tissues of Snell dwarf mice and growth hormone receptor-null mice are not broadly upregulated (Swindell et al., 2009). As mRNA levels often correlate poorly with protein levels (e.g. Ideker et al., 2001; Le Naour et al., 2001), it was important to confirm the absence of a broad and systematic Hsp upregulation by direct quantification of protein levels. The lack of relationship is surprising. One characteristic of Snell dwarf mice is reduced IGF-1 signalling, which is relatively robustly associated with increased Hsp

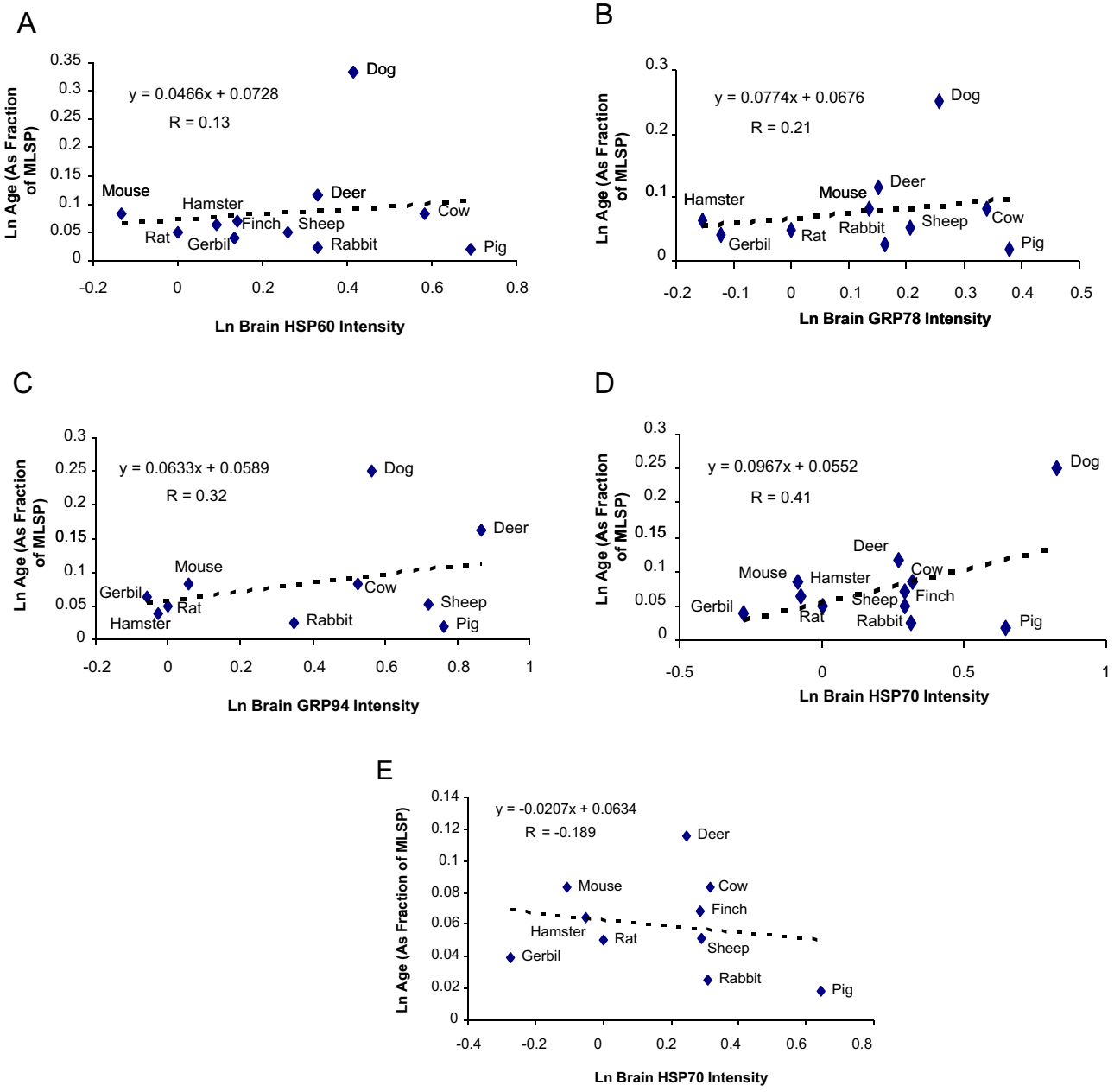

Fig. 6. Hsp levels did not correlate with animal age as a fraction of MLSP. Shown are (A) Hsp60, (B) GRP78, (C) GRP94 and (D) Hsp70 protein levels in brain tissue (summary statistics for correlations in other tissues are given in Table 4). (E) Shows the same analysis as (D) but with the dog data point omitted. All values are natural-log transformed and each data point represents the mean from duplicate measurements made on samples from 3 to 8 individuals of a given species.

expression in invertebrates (Walker and Lithgow, 2003; Halaschek-Wiener et al., 2005). Similarly, a recent report (Stuart and Page, 2010) indicates that plasma IGF-1 levels are lower in larger mammalian species, which are typically longer-lived. This may contribute to the elevated expression of Hsps in longer-lived species. In any event, these divergent results suggest a different relationship between IGF-1 signalling status and longevity in mice than in invertebrates or an inter-species context. Further investigation will be required to understand why this is so.

In conclusion, our study shows strong correlations of Hsps with longevity in mammalian and avian species, suggesting that one mechanism underlying the evolution of longevity has been improved protein homeostasis via increased constitutive expression of Hsps. In contrast, we found no evidence for increased Hsp expression in tissues of long-lived Snell dwarf mice, suggesting no role for constitutive Hsp expression in this model of lifespan extension.

References

Ali, Y., Kitay, B., Zhai, R., 2010. Dealing with misfolded proteins: examining the neuroprotective role of molecular chaperones in neurodegeneration. Molecules 15, 6859–6887.

Bender, A., Hajieva, P., Moosmann, B., 2008. Adaptive antioxidant methionine accumulation in respiratory chain complexes explains the use of a deviant genetic code in mitochondria. Proc. Natl. Acad. Sci. U.S.A. 105, 16496–16501.

Bukau, B., Weissman, J., Horwich, A., 2006. Molecular chaperones and protein quality control. Cell 125, 443–451.

Calderwood, S., Murshid, A., Prince, T., 2009. The shock of aging: molecular chaperones and the heat shock response in longevity and aging. Gerontology 55, 550–558.

Chen, B., Zhong, D., Monteiro, A., 2006. Comparative genomics and evolution of the HSP90 family of genes across all kingdoms of organisms. BMC Genom. 7, 1–19.

Corydon, T.J., Hansen, J., Bross, P., Jensen, T.G., 2005. Down-regulation of HSP60 expression by RNAi impairs folding of medium-chain acyl-CoA dehydrogenase wild-type and disease-associated proteins. Mol. Genet. Metab. 85, 260–270.
Daugaard, M., Rohde, M., Jäättelä, M., 2007. The heat shock protein 70 family: highly homologous proteins with overlapping and distinct functions. FEBS Lett. 581, 3702–3710.
De Magalhaes, J.P., Costa, J., Toussaint, O., 2005. HAGR: the human ageing genomic resources. Nucleic Acids Res. 33, D537–D543.
Dedmon, M.M., Christodoulou, J., Wilson, M.R., Dobson, C.M., 2005. Heat shock protein 70 inhibits alpha-synuclein fibril formation via preferential binding to prefibrillar species. J. Biol. Chem. 280, 14733–14740.
Dou, F., Netzer, W., Tanemura, K., Li, F., Hartl, F.U., Takashima, A., Gouras, G., Greengard, P., Xu, H., 2003. Chaperones increase association of tau protein with microtubules. Proc. Natl. Acad. Sci. U.S.A. 100, 721–726.
Dukes, A.A., Van Laar, V.S., Cascio, M., Hastings, T.G., 2008. Changes in endoplasmic reticulum stress proteins and aldolase A in cells exposed to dopamine. J. Neurochem. 106, 326–333.
Felsenstein, J., 1985. Phylogenies and the comparative method. Am. Nat. 125, 1–15.
Gifondorwa, D., Robinson, M., Hayes, C., Taylor, A., Prevette, D., Oppenheim, R., Caress, J., Milligan, C., 2007. Exogenous delivery of heat shock protein 70 increases lifespan in a mouse model of amyotrophic lateral sclerosis. J. Neurosci. 27, 13173–13180.
Gorbunova, V., Bozzella, M.J., Seluanov, A., 2008. Rodents for comparative aging studies: from mice to beavers. Age 30, 111–119.
Hackett, S.J., Kimball, R.T., Reddy, S., Bowie, R.C.K., Braun, E.L., Braun, M.J., Chojnowski, J.L., Cox, W.A., Han, K.L., Harshman, J., Huddleston, C.J., Marks, B.D., Miglia, K.J., Moore, W.S., Sheldon, F.H., Steadman, D.W., Witt, C.C., Yuri, T., 2008. A phylogenomic study of birds reveals their evolutionary history. Science 320, 1763–1768.
Halaschek-Wiener, J., Khattra, J.S., McKay, S., Pouzyrev, A., Stott, J.M., Yang, G.S., Holt, R.A., Jones, S.J., Marra, M.A., Brooks-Wilson, A.R., Riddle, D.L., 2005. Analysis of long-lived *C. elegans* daf-2 mutants using serial analysis of gene expression. Genome Res. 15, 603–615.
Hayashida, N., Fujimoto, M., Tan, K., Prakasam, R., Shinkawa, T., Li, L., Ichikawa, H., Takii, R., Nakai, A., 2010. Heat shock factor 1 ameliorates proteotoxicity in cooperation with the transcription factor NFAT. EMBO J. 29, 3459–3469.
Hsu, A., Murphy, C., Kenyon, C., 2003. Regulation of aging and age-related disease by daf-16 and heat shock factor. Science 300, 1142–1145.
Ideker, T., Thorsson, V., Ranish, J., Christmas, R., Buhler, J., Eng, J., Bumgarner, R., Goodlett, D., Aebersold, R., Hood, L., 2001. Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. Science 292, 929–934.
Kapahi, P., Boulton, M.E., Kirkwood, T., 1999. Positive correlation between mammalian life span and cellular resistance to stress. Free Radic. Biol. Med. 26, 495–500.
Kirkwood, T., Kapahi, P., Shanley, D., 2000. Evolution, stress and longevity. J. Anat. 197, 587–590.
Kudo, T., Kanemoto, S., Hara, H., Morimoto, N., Morihara, T., Kimura, R., Tabira, T., Imaizumi, K., Takeda, M., 2008. A molecular chaperone inducer protects neurons from ER stress. Cell Death Differ. 15, 364–375.
Le Naour, F., Hohenkirk, L., Grolleau, A., Misek, D., Lescure, P., Geiger, J., Hanash, S., Beretta, L., 2001. Profiling changes in gene expression during differentiation and maturation of monocyte-derived dendritic cells using both oligonucleotide microarrays and proteomics. J. Biol. Chem. 276, 17920–17931.
Lee, A.S., 2005. The ER chaperone and signaling regulator GRP78/BiP as a monitor of endoplasmic reticulum stress. Methods 35, 373–381.
Magen, D., Georgopoulos, C., Bross, P., Ang, D., Segev, Y., Goldsher, D., Nemirovski, A., Shahar, E., Ravid, S., Luder, A., Heno, B., Gershoni-Baruch, R., Skorecki, K., Mandel, H., 2008. Mitochondrial hsp60 chaperonopathy causes an autosomal-recessive neurodegenerative disorder linked to brain hypomyelination and leukodystrophy. Am. J. Hum. Genet. 83, 30–42.
McArdle, A., Dillmann, W., Mestril, R., Faulkner, J.A., Jackson, M., 2004. Overexpression of HSP70 in mouse skeletal muscle protects against muscle damage and age-related muscle dysfunction. FASEB J. 18, 355–357.
McLean, P.J., Kawamata, H., Shariff, S., Hewett, J., Sharma, N., Ueda, K., Breakefield, X.O., Hyman, B.T., 2002. TorsinA and heat shock proteins act as molecular chaperones: suppression of alphasynuclein aggregation. J. Neurochem. 83, 846–854.
Miller, R.A., Williams, J., Kiklevich, J.V., Austad, S., Harper, J.M., 2011. Comparative cellular biogerontology: primer and prospectus. Ageing Res. Rev. 10, 181–190.
Moosmann, B., Behl, C., 2008. Mitochondrially encoded cysteine predicts animal lifespan. Aging Cell 7, 32–46.
Morrow, G., Kim, H.J., Le Pécheur, M., Kaul, S.C., Wadhwa, R., Tanguay, R.T., 2010. Protection from aging by small chaperones: a trade-off with cancer? Ann. NY. Acad. Sci. 1197, 67–75.
Ogburn, C., Carlberg, K., Ottinger, M.A., Holmes, D., Martin, G.M., Austad, S.N., 2001. Exceptional cellular resistance to oxidative damage in long-lived birds requires active gene expression. J. Geronto. (Bio. Sci.) 56A, B468–B474.
Page, M.M., Peters, C.W., Staples, J.F., Stuart, J.A., 2009. Intracellular antioxidant enzymes are not globally upregulated during hibernation in the major oxidative tissues of the 13-lined ground squirrel *Spermophilus tridecemlineatus*. Comp. Biochem. Physiol. A: Mol. Integr. Physiol. 152, 115–122.
Page, M.M., Richardson, J., Wiens, B., Tiedtke, E., Peters, C., Faure, P., Burness, G., Stuart, J.A., 2010. Antioxidant enzyme activities are not broadly correlated with longevity in 14 vertebrate endotherm species. Age 32, 255–270.
Perez, V., Buffenstein, R., Masamsetti, V., Leonard, S., Salmon, A., Mele, J., Andziak, B., Yang, T., Edrey, Y., Friguet, B., Ward, W., Richardson, A., Chaudhuri, A., 2009. Protein stability and resistance to oxidative stress are determinants of longevity in the longest-living rodent, the naked mole rat. Proc. Natl. Acad. Sci. U.S.A. 106, 3059–3064.
Prasad, A.B., Allard, M.W., NISC Comparative Sequencing Program, Green ED, 2008. Confirming the phylogeny of mammals by use of large comparative sequence data sets. Mol. Biol. Evol. 25, 1795–1808.
Price, T., 1997. Correlated evolution and independent contrasts. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 352, 519–529.
Salmon, A., Leonard, S., Masamsetti, V., Pierce, A., Podlutsky, A., Podlutskaya, N., Richardson, A., Austud, S., Chaudhuri, A., 2009. The long lifespan of two bat species is correlated with resistance to protein oxidation and enhanced protein homeostasis. FASEB J. 23, 2317–2326.
Salway, K.D., Page, M.M., Faure, P.A., Burness, G., Stuart, J., 2010. Enhanced protein repair and recycling are not correlated with longevity in 15 vertebrate endotherm species. Age published online.
Springer, M.S., Murphy, W.J., 2007. Mammalian evolution and biomedicine: new views from phylogeny. Biol. Rev. 82, 375–392.
Steele, A., Hutter, G., Jackson, W., Heppner, F., Borkowski, A., King, O., Raymond, G., Aguzzi, A., Lindquist, S., 2008. Heat shock factor 1 regulates lifespan as distinct from disease onset in prion disease. Proc. Natl. Acad. Sci. U.S.A. 105, 13626–13631.
Stuart, G.W., Moffett, K., Leader, J.J., 2002. A comprehensive vertebrate phylogeny using vector representations of protein sequences from whole genomes. Mol. Biol. Evol. 19, 554–562.
Stuart, J.A., Page, M.M., 2010. Plasma IGF-1 is negatively correlated with body mass in a comparison of 36 mammalian species. Mech. Ageing Dev. 131, 591–598.
Swindell, W.R., 2009. Heat shock proteins in long-lived worms and mice with insulin/insulin-like signaling mutations. Aging 1, 573–577.
Swindell, W.R., Masternak, M.M., Kopchick, J.J., Conover, C.A., Bartke, A., Miller, R.A., 2009. Endocrine regulation of heat shock protein mRNA levels in long-lived dwarf mice. Mech. Ageing Dev. 130, 393–400.
Vanhooren, V., Liu, X., Desmyter, L., Fan, Y., Vanwalleghem, L., Molle, W., Dewaele, S., Praet, M., Contreras, R., Libert, C., Chen, C., 2008. Over-expression of heat shock protein 70 in mice is associated with growth retardation, tumor formation, and early death. Rejuvenation Res. 11, 1013–1020.
Walker, G.A., Lithgow, G.J., 2003. Lifespan extension in C. elegans by a molecular chaperone dependent upon insulin-like signals. Aging Cell 2, 131–139.
Yokoyama, K., Fukumoto, K., Murakami, T., Harada, S., Hosono, R., Wadhwa, R., Mitsui, Y., Ohkuma, S., 2002. Extended longevity of Caenorhabditis elegans by knocking in extra copies of hsp70F, a homolog of mot-2 (mortalin)/mthsp70/Grp75. FEBS Lett. 516, 53–57.
Yoo, B.C., Seidl, R., Cairns, N., Lubec, G., 1999. Heat-shock protein 70 levels in brain of patients with down syndrome and Alzheimer's disease. J. Neural Transm. Suppl. 57, 315–322.
Zhao, Q., Wang, J., Levichkin, I., Stasinopoulos, S., Ryan, M., Hoogenraad, N., 2002. A mitochondrial specific stress response in mammalian cells. EMBO J. 21, 4411–4419.