Mechanisms of Ageing and Development xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

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

Relationship between heat shock protein 70 expression and life span in *Daphnia*

Q1 Charles Schumpert, Indhira Handy, Jeffry L. Dudycha, Rekha C. Patel *

Q2 *Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, United States*

ARTICLE INFO

Article history:
Received 6 September 2013
Received in revised form 23 April 2014
Accepted 29 April 2014
Available online xxx

Keywords:
Daphnia
Heat shock
Hsp70
HSF-1
Longevity
Aging

ABSTRACT

The longevity of an organism is directly related to its ability to effectively cope with cellular stress. Heat shock response (HSR) protects the cells against accumulation of damaged proteins after exposure to elevated temperatures and also in aging cells. To understand the role of Hsp70 in regulating life span of *Daphnia*, we examined the expression of Hsp70 in two ecotypes that exhibit strikingly different life spans. *Daphnia pulicaria*, the long lived ecotype, showed a robust Hsp70 induction as compared to the shorter lived *Daphnia pulex*. Interestingly, the short-lived *D. pulex* isolates showed no induction of Hsp70 at the mid point in their life span. In contrast to this, the long-lived *D. pulicaria* continued to induce Hsp70 expression at an equivalent age. We further show that the Hsp70 expression was induced at transcriptional level in response to heat shock. The transcription factor responsible for Hsp70 induction, heat shock factor-1 (HSF-1), although present in aged organisms did not exhibit DNA-binding capability. Thus, the decline of Hsp70 induction in old organisms could be attributed to a decline in HSF-1's DNA-binding activity. These results for the first time, present a molecular analysis of the relationship between HSR and life span in *Daphnia*.

1. Introduction

Aging is a universal property of multicellular organisms that causes functional decline of all biological systems. Proteotoxicity due to misfolded proteins is a central component of aging and the ability of organisms to deal with misfolded proteins is crucial for longevity (Taylor and Dillin, 2011). As an organism ages, the free radicals increase within cells due to mitochondrial malfunctions and inefficiency, which in turn leads to an increase in altered proteins (Cui et al., 2012). The pathologies and phenotypes of aging are caused primarily by the inability to deal with proteotoxic stress and an accumulation of altered proteins (Clancy and Birdsall, 2013). In order to appropriately respond to proteotoxic stress, a physiological response termed the heat shock response (HSR) is induced, which involves a rapid and transient increase in expression of molecular chaperones such as heat shock proteins (Hsps) following a proteotoxic stimulus (Calderwood et al., 2009). HSR enables an organism to handle proteotoxic conditions and survive without an extended, damaging imbalance in protein homeostasis. Molecular chaperones, such as Hsp70, act to renature the denatured or misfolded proteins or trigger their degradation if renaturation is not possible (Bukau et al., 2006). The Hsp gene expression is induced at transcriptional level and is mainly regulated by the transcription factor Heat Shock Factor-1 (HSF-1) (Anckar and Sistonen, 2011).

Among molecular chaperones, Hsp70 plays a regulatory role in the aging process since it mitigates the effects of proteotoxic stress (Calderwood et al., 2009; Kim et al., 2013). Overexpression of Hsp70 by knocking in more copies of the gene in *Caenorhabditis elegans* led to an overall increase in lifespan (Yokoyama et al., 2002). Consistent with this, knockdown of Hsp70 led to accelerated aging including premature death (Kimura et al., 2007). Hsp70 and its transcriptional regulator HSF-1 are both important in coping with proteotoxic stress as well as the general increase of altered and misfolded proteins in aged organisms (Morley and Morimoto, 2004; Morimoto and Cuervo, 2009; Anckar and Sistonen, 2011). Hsp70 protein levels can be used as a predictor of total life span in *C. elegans* and *Drosophila melanogaster*, because the organisms that display a more robust HSR and a higher expression of Hsp70 also have longer lifespans (Rea et al., 2005; Tower, 2011). In the present

* Corresponding author at: Department of Biological Sciences, University of South Carolina, 700 Sumter Street, Columbia, SC 29208, United States.
Tel.: +1 803 777 1853; fax: +1 803 777 4002.
E-mail address: patelr@biol.sc.edu (R.C. Patel).

http://dx.doi.org/10.1016/j.mad.2014.04.001
0047-6374/

study we have investigated the HSR of two different ecotypes of *Daphnia* that have very different life spans by examining the expression of Hsp70.

Daphnia are small freshwater crustaceans that are an important model system in ecology, evolutionary biology, and ecotoxicology (Benzie, 2005). These organisms are easy to maintain, reproduce via cyclic parthenogenesis, have transparent carapaces, and have relatively short life-span, thus making them a good experimental model. In cyclic parthenogenesis, wild populations reproduce mainly via ameiotic cloning (parthenogenesis), but periodic environmental stress induces sexual reproduction. Parthenogenetic reproduction can be enforced in the lab, making it possible to maintain isogenic individuals from one generation to the next that reflect naturally occurring genomes. These unique properties have made them a useful model for research on naturally occurring patterns of variation in aging (Dudycha, 2001; Dudycha and Hassel, 2013) and on the effect of environmental variation on aging (Dudycha, 2003; Steinberg et al., 2010). We focus on two ecotypes of the *Daphnia pulex* species that are adapted to distinct habitats, and thus have evolved sharply divergent lifespans (Dudycha and Tessier, 1999). *D. pulex* inhabits temporary ponds, where it faces high extrinsic mortality risk and has a short lifespan with an average being about 25–30 days (Dudycha, 2001, 2004). In contrast, *Daphnia pulicaria* inhabits large stratified lakes, encounters low extrinsic mortality, and has a long lifespan with an average being about 60–65 days (Dudycha, 2001, 2004). Though the ecotypes have different names, they are not fully distinct species, and abundant evidence supports ongoing genetic exchange (Dudycha, 2004; Cristescu et al., 2012). Estimated divergence time is only ~82,000 years (Omilian and Lynch, 2009), a relatively short evolutionary span that is lower than the divergence among some human populations. The complete genome sequence of *D. pulex* was recently published (Colbourne et al., 2011). For simplicity, throughout the manuscript, we use the specific terminology *D. pulex* and *D. pulicaria* to distinguish the pond- and lake-ecotypes with short and long life spans respectively. All of the clones used in this study have been acclimated to the lab for >3 years (i.e., >75 asexual generations).

It is clear that these named taxa, though ecologically separate, are not evolutionarily distinct species, and gene flow continues to occur between the habitats at a local scale. Heier and Dudycha (2009) showed that experimental crosses between the ecotypes were fully fertile, and recent population genetic and genome-scale analyses confirm that gene flow and introgression is widespread (Daniel et al., 1995; Cowan et al., 1996; Vergilino et al., 2011; Cristescu et al., 2012; Tucker et al., 2013; Xu et al., 2013). The two ecotypes face different patterns of temperature variation in nature, but the ranges of temperatures they experience are similar. In a study of field demography (Dudycha, 2004) populations in southwest Michigan, including the sources of our study clones, were monitored for a year, confirming that these populations experience similar temperature ranges. In both habitats, *Daphnia* will experience temperatures ranging from 4 °C to the >28 °C, but the physics of water bodies dictates that they experience them differently (Wetzel, 2001). In temporary ponds, the largest change is seasonal, with cold temperatures as snow melts shortly before the population emerges from overwintering dormant eggs, and warmer temperatures as the population produces dormant eggs in summer. There is some daily variation with changes in air temperature, but small ponds are primarily insulated by and reflect ground temperatures. Lakes also have seasonal dynamics, but the populations are active year round, and in winter experience a continuous temperature of 4 °C. By summer, lakes stratify into a warm upper layer (typically ranging from 24 °C to 28 °C) and a cool bottom layer (~10 °C), with *Daphnia* migrating between the two temperature zones on a daily basis.

Previous reports have shown that despite the limited genetic divergence between the ecotypes that there is substantial differentiation of life history and related traits. They differ in lifespan, age-specific mortality rates, the timing and rate of age-dependent increases in mortality rates, maximum reproductive rate, the rate of reproductive decline with aging, and juvenile growth rate (Dudycha and Tessier, 1999). This study included naturally occurring hybrids between the ecotypes which showed intermediate characteristics, and the results were confirmed in later studies (Dudycha, 2001, 2003). Other direct comparisons have indicated there are differences in investment in dormancy and sexual reproduction (Caceres and Tessier, 2011). Physiological studies on *Daphnia* are very few in number; we are unaware of any comparisons of respiration or metabolic rate, though there is limited evidence that *D. pulicaria* has superior photoenzymatic repair of DNA damage (Connelly et al., 2009).

In our present work we establish a foundation for the use of *Daphnia* as a model system in molecular biology of aging. We evaluate age-dependence of the HSR in *D. pulex* and *D. pulicaria*, testing its relationship to their life span. Our results show that the two ecotypes respond differently to heat stress, as measured by analysis of Hsp70 protein expression. *D. pulicaria*, the long lived ecotype, responds better than the shorter lived *D. pulex*, supporting a role for HSR in differential aging of the ecotypes. For the first time, these results present a molecular analysis of the relationship between stress response and regulation of life span in *Daphnia*. In addition, our findings offer a mechanistic insight for the lack of HSR in older organisms by demonstrating that the transcription factor HSF-1 responsible for transcriptional induction of Hsp70 loses its ability to bind DNA as *Daphnia* age.

2. Materials and methods

2.1. Daphnia *cultures*

D. pulex and *D. pulicaria* ecotypes used in this study were isolated from ponds in southwest Michigan, USA in 2008 (except for clone "TCO" which was isolated from Oregon, USA) and have since been cultured in the lab. *Daphnia* are maintained at a temperature of 20 °C with a photoperiod of 12:12 L:D (12 h of light followed by 12 h of dark) within a Percival growth chamber. *Daphnia* were maintained at a concentration of 3–5 animals per 250 ml beaker in 200 ml of filtered lake water. *Daphnia* were cleared of young and transferred to a new beaker with fresh water on alternate days. They were fed every day with vitamin-supplemented algae *Ankistrodesmus falcatus* at a concentration of 20,000 cells/ml. To generate experimental animals, even-aged cohorts were begun by placing neonates individually in 100 ml of filtered lake water. Experimental animals were otherwise maintained as in the source cultures.

2.2. Heat shock treatment

Daphnia of specific ages were placed into groups of 25–30 organisms in 1 ml of lake water in a microcentrifuge tube. The tubes were placed in a heating block at 32 °C for 30 min. *Daphnia* were allowed to recover at room temperature (22° C) for 4 or 6 h. The control samples were also separated into groups of 25–30 but were kept at room temperature for the duration of the experiment and then harvested.

2.3. Western blot analysis

D. pulex and *D. pulicaria* total protein extracts were prepared from either treated or untreated organisms. All water was removed from the tubes containing *Daphnia*, the organisms were washed once with 1 ml cold PBS. The PBS was removed and the organisms were homogenized using a tight-fitting pestle in RIPA buffer (150 mM NaCl, 1.0% IGEPAL, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0) containing protease inhibitor cocktails (Sigma and Calbiochem). The samples were chilled on ice for 5 min, centrifuged at 13,000 × *g* for 4 min and supernatant was saved as total protein extract. The protein concentration was determined immediately using the BCA kit (Pierce), Laemmli's sample loading buffer (4×) was immediately added to the extract following protein concentration determination and samples were heated at 95 °C for 5 min to further inactivate any residual proteases from the *Daphnia*. This protocol ensures efficient inactivation of gut proteases. 50 μg of total protein was separated by SDS-PAGE and a western blot analysis was performed using ECL plus (Pierce) chemifluorescence detection. The primary antibodies used are as follows: Hsp70 (Fisher MAB3-007, 1:1000), β-actin (Sigma A5441, clone AC-15, 1:5000), α-tubulin (Sigma, T5168, 1:5000).

Please cite this article in press as: Schumpert, C., et al., Relationship between heat shock protein 70 expression and life span in *Daphnia*. Mech. Ageing Dev. (2014), http://dx.doi.org/10.1016/j.mad.2014.04.001