**ISSN** 1672-9145 Acta Biochimica et Biophysica Sinica 2004, **36**(9): 618–622 **CN** 31-1940/Q

# Trichostatin A Extends the Lifespan of *Drosophila melanogaster* by Elevating *hsp22* Expression

Dan TAO[#], Jun LU[#], Hui SUN, Yan-Mei ZHAO, Zhi-Gen YUAN, Xiao-Xue LI, and Bai-Qu HUANG*

*Institute of Genetics and Cytology, Northeast Normal University, Changchun 130024, China*

**Abstract** The level of acetylation of histones in nucleosomes is related to the longevity of yeast and animals. However, the mechanisms by which acetylation and deacetylation affect longevity remain unclear. In present study, we investigated the influence of histone acetylation modification on the expression of *hsp22* gene and the lifespan in *Drosophila melanogaster* using histone deacetylase (HDAC) inhibitor Trichostatin A (TSA). The results showed that TSA could extend the lifespan of *Drosophila melanogaster*. Furthermore, TSA significantly promoted the *hsp22* gene transcription, and affected the chromatin morphology at the locus of *hsp22* gene along the polytene chromosome. Present data implicate that TSA may affect the lifespan of *Drosophila* through changing the level of histone acetylation and influencing the expression of *hsp22* gene that is related to aging.

**Key words** histone acetylation; histone deacetylase inhibitor; *Drosophila melanogaster*; *hsp22*; lifespan

Aging can be defined as time-dependent, gradual and detrimental changes in the structure and physiological function of an organism, ultimately leading to death [1]. Factors influencing the aging process would change the longevity. In *Drosophila melanogaster*, several mutations have been shown to extend the lifespan, e.g., *methuselah* encoding a putative G-protein coupled receptor [2], *Indy* encoding a sodium dicarboxylate co-transporter [3], *chico* encoding insulin receptor substrate [4] and *InR* encoding the insulin-like receptor [5]. Extended longevity phenotypes were also observed in transgenic flies overexpressing antioxidant enzymes Cu/Zn superoxide dismutase and catalase, or a molecular chaperone Hsp70.

Histone acetylation has been shown to be related to longevity. The histone deacetylases (HDACs) SIR2 and RPD3 could change the longevity because the deletion of *sir2* shortened the lifespan [6] while *rpd3* knockout prolonged the lifespan in *Saccharomyces cerevisiae* [7]. Furthermore, overexpression of SIR2 ex-tended the budding yeast lifespan [6]. Treatment with PBA (4-phenylbutyrate, a kind of HDAC inhibitor) resulted in histone hyperacetylation and lifespan extension in *Drosophila* [8]. However, the roles of histone deacetylases in aging and longevity remain unknown.

Post-translational acetylation of the specific lysine residues in the amino terminus of histones by histone acetyltransferases (HATs) is thought to neutralize the positive charges to generate a more open DNA conformation to allow the access of transcriptional factors to the target genes. Deacetylation of histones by HDACs, on the other hand, restores the positive charges on histones by removing the acetyl groups and leads to the condensation of nucleosome structure. This process may determine the specific temporal and spatial gene expression patterns in animals. Thus, it is important to investigate the physiological changes in animals brought about by the alteration of the level of histone acetylation, which can be studied by using HDAC inhibitors. The HDAC inhibitors commonly used are TSA (Trichostatin A), BuA (sodium butyrate) and PBA, etc.. The activity of RPD3 can be blocked by HDAC inhibitor TSA [9].

Aging is associated with the accumulation of non-active enzymes and proteins with partial degradation

Received: Match 26, 2004 Accepted: July 2, 2004

This work was supported by the grants from the National Natural Science Foundation of China (30370316) and the Major State Basic Research Project of China (G1999053902)

[#]These authors contributed equally to this work

*Corresponding author: Tel, 86-431-5099768; Fax, 86-431-5681833; E-mail, huangbq@nenu.edu.cn

and/or oxidative damage. Proteins with antioxidant or repairing function usually have anti-aging effects [10]. The heat shock proteins (HSPs) are members of a large family of molecular chaperones, characterized by their abilities to affect the structure or folding of other proteins. They are induced to express in response to protein damage caused by heat and other stresses [11–14]. Our previous study showed that HDAC inhibitor TSA promoted the transcription intensity of *hsp70* gene in *Drosophila* [15], suggesting that histone acetylation modification might play a role in *hsp* genes regulation.

The *hsp22* gene has the lowest expression level during development compared with other *hsp*s, but gradually reaches the highest expression level with aging. Meanwhile, the expression of *hsp22* is regulated at both transcriptional and posttranscriptional level in *Drosophila* [16]. The dramatic induction of *Drosophila hsp22* RNA during aging appears to be a novel pattern of gene expression in higher eukaryotes [16].

In this study, the correlation of the histone acetylation, *hsp22* expression and the lifespan in *Drosophila* was investigated. The data of the present work showed that changes in acetylation level mediated by HDAC inhibitor played an important role in promoting the transcription of *hsp22* gene and in the extension of lifespan in *Drosophila*.

## Materials and Methods

### Fly strain and HDAC inhibitor

The wild-type *Drosophila melanogaster* strain Canton-S was used. The HDAC inhibitor Trichostatin A (TSA) was purchased from Sigma (USA). TSA dissolved in physiological saline was added in standard medium at a final concentration of 10 μM.

### Longevity determination

Newly eclosed flies were collected and raised in standard medium, transferred to fresh vials every 3 d, and maintained at 29 °C. The number of dead flies was counted everyday. Both the TSA treated and control group consisted of two subgroups (female and male).

### Observation of the morphology of polytene chromosome

The third instar larvae of *Drosophila melanogaster* with or without TSA treatment were used. Salivary glands were dissected, stained with fuchsin and examined under a light microscope [15].

### The estimation of *hsp22* expression

The semi-quantitative determination of Hsp22 mRNA was accomplished by RT-PCR with sense primer 5′-CTTTCACGCCTTCTTCC-3′ and antisense primer 5′-GCGGTTTTGTCTTTTGG-3′. Primers for *rp49* gene (a house-keeping gene used as internal reference) were sense primer 5′-AGCACTTCATCCGCCACC-3′ and antisense primer 5′-ATCTCGCCGCAGTAAACG-3′.

The total RNA was extracted from the head and trunk of flies respectively at day 6, 12, 18, 26 and day 33 post eclosion using RNA extraction kit (Promega). RT-PCR was carried out using an RT-PCR system (Promega) following the manufacturer's protocol. The estimation of the RT-PCR products was done by photodensitometric analysis of the bands in agarose gel after electrophoresis, and the results were presented as the relative ratio of intensity values between *hsp22* and *rp49* bands.

## Results

### Influence of HDAC inhibitor TSA on the lifespan of *Drosophila*

We estimated the effect of TSA on the longevity of female and male flies respectively at 29 °C. As shown in Fig. 1, TSA treatment extended the mean survival time significantly by 27.3% for female fly [Fig. 1(A)] and 23.3% for male fly [Fig. 1(B)]. Meanwhile, TSA also significantly extended the maximum survival time by 37.9% for female fly [Fig. 1(A)] and by 37.0% for male fly [Fig. 1(B)]. The lifespan of the female fly was slightly longer than that of male one, which might be due to that the female had the higher tolerance to the stresses during aging.

### Changes in morphology of polytene chromosome after TSA treatment

The polytene chromosome of *Drosophila* is a good model for studying the relationship between gene activity and chromosome structure. The "puff" structures that appear at the defined sites along the chromosome have long been thought to represent the loci of activated genes [18], and it is believed that the size of the "puff" is positively correlated with the transcription intensity of the corresponding gene. We examined the morphology at the chromosome site where *hsp22* gene is located after the TSA treatment. Under normal physiological conditions, no apparent puff structure was detected at the *hsp22* gene

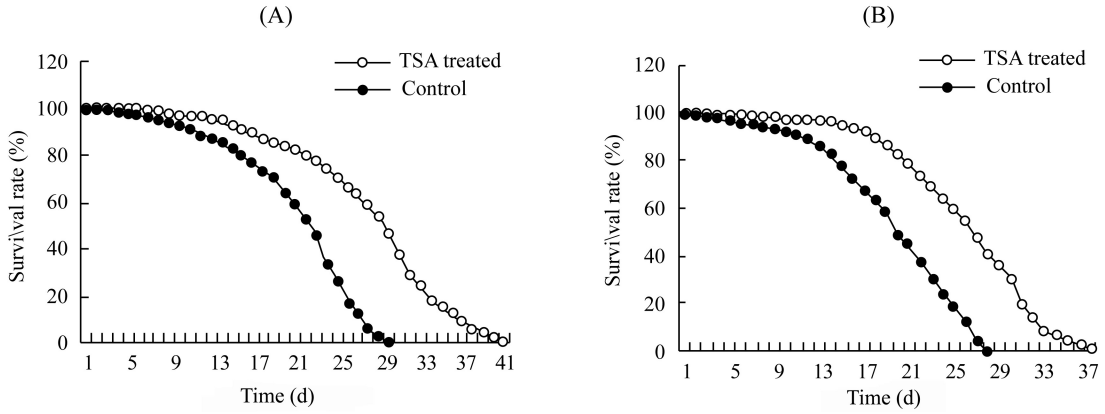


**Fig. 1 Effects of TSA on the lifespan of *Drosophila***

The mean survival time was extended by 27.3% for female and 23.3% for male, respectively. Maximum survival time was extended by 37.9% for female and 37.0% for male, respectively. The difference of maximum survival time between TSA treatment and control was very significant ($P$<0.01). (A) Female fly group. (B) Male fly group.

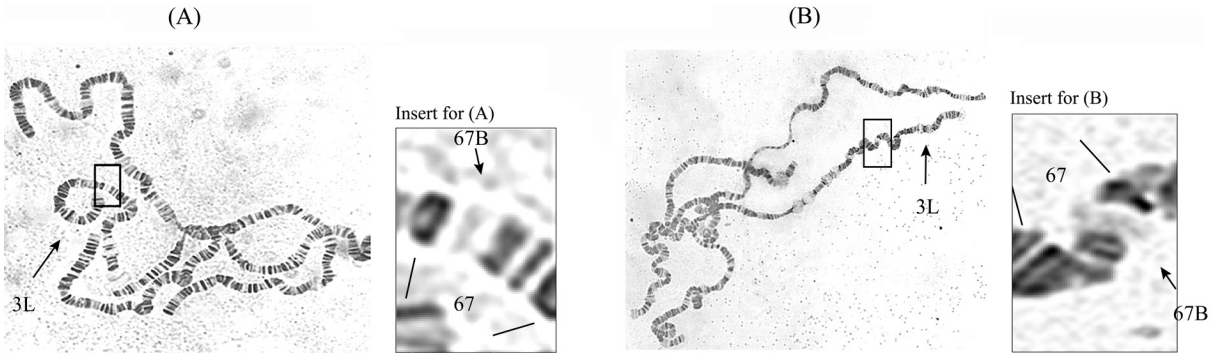


**Fig. 2 Changes in morphology of polytene chromosome induced by TSA**

(A) Polytene chromosomes from the larvae of control group. (B) Polytene chromosomes from the larvae with TSA treatment. The smaller picture at the right was the magnified view of the boxed area in left picture. The number "67" indicated the region of chromosome 3L of *Drosophila*, and the arrows denote the "puff" structure corresponding to the *hsp22* gene locus at region 67.

locus (3L67B) [Fig. 2(A)], presumably due to the low basal transcription level of *hsp22* gene. While in the TSA treated groups, puff structure could be detected at the *hsp22* locus [Fig. 2(B)].

### Changes of *hsp22* gene transcription induced by TSA

To determine whether the TSA-mediated chromatin structure change was caused by the up-regulation of *hsp22* gene expression, semi-quantitative RT-PCR amplification was used to analyze the change of *hsp22* gene transcription. The RT-PCR products were run on agarose gel and quantified by photodensitometry. The ratio between the density reading of *hsp22* gene and *rp49* gene bands was taken. As we could see from Fig. 3, TSA promoted *hsp22* transcriptional level at different day post eclosion compared with the control. The level of *hsp22* transcription in fly head with TSA treatment at day 6 was not significantly different from that of the control (Fig. 3I). The differences between the TSA treatment and control groups at days 12, 18 and day 26 were significant ($P$<0.05) and very significant ($P$<0.01), respectively (Fig. 3I). The above results indicated that TSA advanced *hsp22* transcription in heads, and this augmentation was increased with the time after eclosion. However, the difference at day 33 was less prominent (Fig. 3I), probably due to *hsp22* expression had approached the limit.

A similar pattern of *hsp22* expression was observed in trunks. The level of *hsp22* transcription in trunks with TSA treatment at day 6 was not significantly different from that of control, whereas the differences at day 12 and day 18 became significant ($P$<0.05) and very significant ($P$<0.01), respectively (Fig. 3II). The difference between the TSA

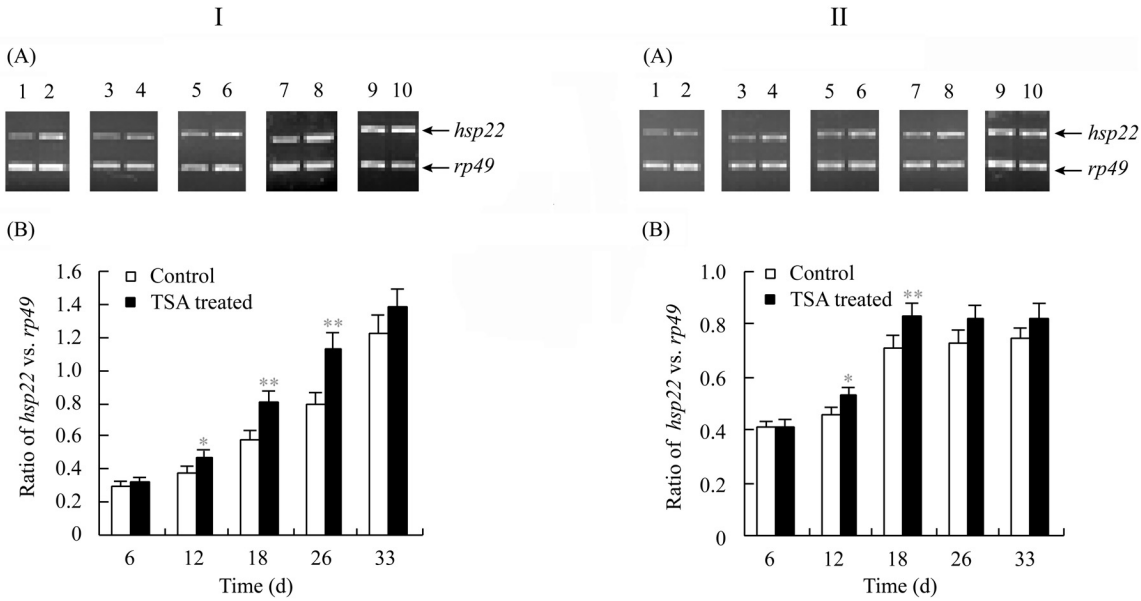


**Fig. 3 Semi-quantitative RT-PCR analysis of *hsp22* gene expression in the head and trunk of fly**

I: fly head *hsp22* gene transcription level analysis. (A) Agarose gel electrophoresis result. 1, 3, 5, 7 and 9, are controls of flies at day 6, 12, 18, 26 and 33 post eclosion, respectively; 2, 4, 6, 8 and 10, 10 μM TSA treated fly at day 6, 12, 18, 26 and 33, respectively. (B) Photodensitometric analysis result. II: fly trunk *hsp22* gene transcription level analysis. (A) Agarose gel electrophoresis result. 1, 3, 5, 7 and 9, are controls of flies at day 6, 12, 18, 26 and 33 post eclosion, respectively; 2, 4, 6, 8 and 10, 10 μM TSA treated fly at day 6, 12, 18, 26 and 33, respectively. (B) Photodensitometric analysis result. **P*<0.05 vs. control; ***P*<0.01 vs. control.

treatment and control at day 26 and day 33 was insignificant (Fig. 3II). Comparing with the data from the heads, the extent of increases between TSA treatment and control at different days in trunks were slight, indicating that the difference of *hsp22* expression between head and other parts of the body existed. The reason for this difference may be that there are more abundant nerve cells in head than in other parts of body, and the processes associated with aging and longevity are more closely related to nervous system.

## Discussion

The present work showed that HDAC inhibitor TSA extended the *Drosophila* lifespan, and this was presumably due to the inhibition of the activity of certain HDACs. The activity of RPD3 (a kind of HDAC) could be blocked by TSA [9] and *rpd3* deletion extended lifespan in *Saccharomyces cerevisiae* [7]. As a result, repression of the activity of RPD3 might activate or promote the expression of relevant genes (including *hsp*s). A similar mechanism may exist in *Drosophila*. It has been reported that another HDAC inhibitor PBA can extend the *Drosophila* lifespan [8]. It has also been known that molecular chaperones play important roles in maintaining cellular functions during aging, through promoting protein renaturation and preventing proteins from aggregation and denaturation [19,20]. The increase of the expression of chaperones may inhibit the accumulation of damage, and/or stimulate repair mechanisms, raise the resistance of cell to stresses to create a favorable physiological and cellular environment for longevity.

In order to reveal the molecular mechanisms of extension of *Drosophila* longevity by HDAC inhibitor TSA, we analyzed the changes of *hsp22*, a gene related to aging process, at the chromosome structure level and mRNA level. The results confirmed that TSA might elevate histone acetylation level by repressing the activity of HDACs, which could loosen the chromatin at particular loci and form puffs. Meanwhile, the expression of genes at these loci may increase.

It was apparent that the significant change of *hsp22* mRNA in both heads and trunks of flies after TSA treatment began at day 12. Compared with the longevity, the survival rate of flies began to reduce remarkably at about day 12, which may be due to the accumulation of deleterious metabolic products *in vivo* at this time, while at the same time the gene (such as *hsp22*) products with the function of detoxification began to act. After TSA

treatment, *hsp22* transcription level increased and HSP22 retarded the initiation of aging resulting to extend *Drosophila* lifespan. However, extension of longevity is not, of course, merely attributable to the change of a single gene expression. TSA can raise the acetylation level of histones, and probably certain transcriptional factors *in vivo*, and promote the expression of particular genes. We also detected changes of morphology at other chromosome sites after TSA treatment (data not shown). The genes at these sites included those encoding antioxidant enzymes, other heat shock proteins and proteins associated with mitochondrial function. However, whether these genes are actually involved in *Drosophila* aging and longevity, and how they function, require more detailed studies.

Present data demonstrated that TSA can extend the *Drosophila* longevity; it can also change the puff morphology at the chromosome site where *hsp22* gene is located and significantly promote the transcription of *hsp22* gene. These results strongly suggest a correlation of the histone acetylation, *hsp22* expression and longevity in *Drosophila*. We therefore proposed here that TSA might extend the lifespan of *Drosophila* through changing the level of histone acetylation, which in turn promotes the expression of *hsp22* gene which lead to affecting the aging and longevity.

## References


1 Arking R. Biology of Aging: Observations and Principles. 2nd ed. Sunderland: Sinauer, 1998

2 Lin YJ, Seroude L, Benzer S. Extended life-span and stress resistance in the *Drosophila* mutant *methuselah*. Science, 1998, 282: 943–946

3 Rogina B, Reenan RA, Nilsen SP, Helfand SL. Extended life-span conferred by co-transporter gene mutations in *Drosophila*. Science, 2000, 290: 2137–2140

4 Clancy DJ, Gems D, Harshman LG, Oldham S, Stocker H, Hafen E, Leevers SJ *et al*. Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. Science, 2001, 292: 104–106

5 Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS. A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. Science, 2001, 292: 107–110

6 Kaeberlein M, Mcvey M, Guarente L. The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. Genes Dev, 1999, 13: 2570–2580

7 Kim S, Benguria A, Lai CY, Jazwinski SM. Modulation of life-span by histone deacetylase genes in *Saccharomyces cerevisiae*. Mol Biol Cell, 1999, 10: 3125–3136

8 Kang HL, Benzer S, Min KT. Life extension in *Drosophila* by feeding a drug. Proc Natl Acad Sci USA, 2002, 99: 838–843

9 Taunton J, Hassig CA, Schreiber SL. A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science, 1996, 272: 408–411

10 Arking R, Burde V, Graves K, Hari R, Feldman E, Zeevi A, Soliman S *et al*. Identical longevity phenotypes are characterized by different patterns of gene expression and oxidative damage. Exp Gerontol, 2000, 35: 353–373

11 Lindquist S. Varying patterns of protein synthesis in *Drosophila* during heat shock: Implications for regulation. Dev Biol, 1980, 77: 463–479

12 Ananthan J, Goldberg AL, Voellmy R. Abnormal proteins serve as eukaryotic stress signals and trigger the activation of heat shock genes. Science, 1986, 232: 522–524

13 Parsell DA, Lindquist S. The function of heat-shock proteins in stress tolerance: Degradation and reactivation of damaged proteins. Annu Rev Genet, 1993, 27: 437–496

14 Morimoto RI, Tissieres A, Georgopoulos C. The Biology of Heat Shock Proteins and Molecular Chaperones. New York: Cold Spring Harbor Laboratory Press, 1994

15 Chen T, Sun H, Lu J, Zhao Y, Tao D, Li X, Huang B. Histone acetylation is involved in *hsp70* gene transcription regulation in *Drosophila melanogaster*. Arch Biochem Biophys, 2002, 408: 171–176

16 King V, Tower J. Aging-specific expression of *Drosophila hsp22*. Dev Biol, 1999, 207: 107–118

17 Wheeler JC, Bieschke ET, Tower J. Muscle-specific expression of *Drosophila hsp70* in response to aging and oxidative stress. Proc Natl Acad Sci USA, 1995, 92: 10408–10412

18 Pelling C. Ribonucleic acid synthesis in giant chromosomes. Autoradiographic investigations on *Chironomus tentans*. Chromosoma, 1964, 15: 71–122

19 Hartl FU. Molecular chaperones in cellular protein folding. Nature, 1996, 381: 571–579

20 Souren JE, Wiegant FA, van Wijk R. The role of *hsp70* in protection and repair of luciferase activity *in vivo*; experimental data and mathematical modelling. Cell Mol Life Sci, 1999, 55: 799–811


Edited by
**You-Shang ZHANG**