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View point

Regulation of lifespan by histone deacetylase

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Abstract

Aging is a universal biological phenomenon in eukaryotes, but why and how we age still remain mysterious. It would be of great biological interest and practical importance if we could uncover the molecular mechanism of aging, and find a way to delay the aging process while maintaining physical and mental strengths of youth. Histone deacetylases (HDACs) such as SIR2 and RPD3 are known to be involved in the extension of lifespan in yeast and *Caenorhabditis elegans*. An inhibitor of HDACs, phenylbutyrate, also can significantly increase the lifespan of *Drosophila*, without diminution of locomotor vigor, resistance to stress, or reproductive ability. Treatment for a limited period, either early or late in adult life, is also effective. Alteration in the pattern of gene expression, including induction or repression of numerous genes involved in longevity by changing the level and the pattern of histone acetylation may be an important factor in determining the longevity of animals. Published by Elsevier Science Ireland Ltd.

Keywords: Lifespan; Histone deacetylase; Aging

1. Introduction

Aging can be defined as time-dependent, gradual and detrimental changes in the physiological function and structure of an organism, which ultimately leads to death (Arking, 1998). Aging and mortality are universal phenomena among living creatures, but our understanding of the mechanisms of aging, particularly of aging in humans, remains limited due to biological complexities. The use of simple model systems, however, is beginning to shed new light on the underlying mechanisms of cellular senescence and aging. Modification of a single gene by genetic manipulation in several models including yeast, *Caenorhabditis elegans*, and *Drosophila* has been shown to extend lifespan. In *Drosophila*, mutations in *methuselah*, a G protein-coupled receptor (Lin et al., 1998), *Indy*, a sodium dicarboxylate co-transporter (Rogina et al., 2000), *chico*, an insulin receptor substrate (Clancy et al., 2001),

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and, *InR*, an insulin-like receptor (Tatar et al., 2001), can increase the lifespan of the fly. Additional copy of *sir2* (Tissenbaum and Guarente, 2001), mutations in *age1* (Friedman and Johnson, 1998), *daf2* (Kenyon et al., 1993; Kimura et al., 1997), *clk1* (Wong et al., 1995), *unc64*, and *unc31* (Ailion et al., 1999), in *C. elegans* also increase the lifespan of the worm. In yeast, *sir*2 and *rpd*3, which encodes histone deacetylases (HDACs), are involved in the extension of the budding yeast lifespan of the mother cell (Guarente and Kenyon, 2000).

The themes emerging from the mutational studies in model organisms are that metabolic control, oxidative damage, restriction of nutrients, and regulation of gene expression are the important physiological processes governing aging. How these processes regulate longevity of the animals, however, remains obscure, particularly in the case of HDAC-dependent changes in longevity. SIR2 and RPD3 are both HDACs, yet deletion of *sir2* shortens lifespan (Kaeberlein et al., 1999) while *rpd3* knock out increases lifespan in *Saccharomyces cerevisiae* (Kim et al., 1999). Furthermore, overexpression of SIR2 extends the budding yeast lifespan (Kaeberlein et al., 1999). These conundrums raise interesting questions about how HDACs can affect gene expression and lifespan.

Here we will discuss the recent developments in the regulation of lifespan by HDACs. We will focus on how histone modifications can affect longevity and discuss the controversies surrounding gene silencing and regulation of lifespan by SIR2 and RPD3. We will also survey the known inhibitors of HDAC and review the effect of one particular HDAC inhibitor, phenylbutyrate, on the extension of lifespan.

2. Histone deacetylases

The acetylation and deacetylation of histones in nucleosomes play an important role in regulating gene expression. In eukaryotic cells, DNA is packaged into chromatin containing nucleosomes as the basic unit. The nucleosome is composed of DNA wrapped around two pairs each of the highly conserved core histones H2A, H2B, H3 and H4. Post-translational acetylation of the specific lysine resides in the amino terminals of histones by histone acetyltransferases (HATs) is thought to neutralize the positive charge, thus, generate a more open DNA conformation to allow the access of transcription factors on 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 condensation of the nucleosome structure (Fig. 1). Hyperacetylated histones are linked to transcriptionally active domains, whereas hypoacetylated histones are generally associated with transcriptionally silent loci (Strahl and Allis, 2000).

There are three different classes of HDACs known thus far (Table 1), identified based on homology of the catalytic domains to yeast HDACs (Gray and Ekström, 2001; Khochbin et al., 2001). Class I HDACs include yeast RPD3, *Drosophila* RPD3, human HDAC1–3, and HDAC8. Class II includes yeast HDA1, human HDAC4–7, and the newly identified HDAC9 and 10 (Zhou et al., 2001; Kao et al., 2002; Guardiola and Yao, 2002). The class II deacetylases are large proteins with molecular weight around 130 kDa and have been shown to be shuttled from the cytoplasm to the nucleus when required. Class III consists of yeast SIR2, *Drosophila* SIR2, *C. elegans* SIR2, and human SIRT1–7. SIR2 protein shows NAD^{+}-dependent HDAC activity (Imai et al., 2000). SIR2 is insensitive to trichostatin

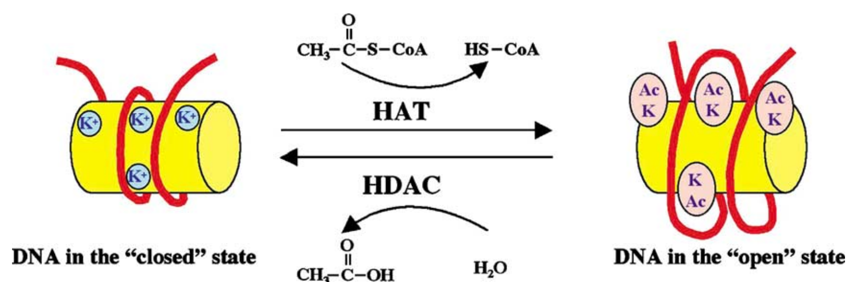

Fig. 1. Schematic diagram depicting alteration in the nucleosome structure due to acetylation by histone acetyltransferase (HAT) and deacetylation by histone deacetylase (HDAC). Nucleosome is composed of core histones (yellow cylinder) and DNA (red line). Acetylation (Ac) on lysine residues (K) can change nucleosomal DNA to the "open" state, which is generally associated with transcriptionally active domains. Deacetylation by HDAC restores the positive charges on lysine residues to condense the nucleosome.

(TSA), an inhibitor of HDACs (Imai et al., 2000), but the activity of RPD3 can be blocked by TSA (Taunton et al., 1996).

Different HDACs recruit various types of co-factors and form distinct nuclear complexes that bring transcription factors to a local region in the chromatin to act as transcriptional repressor (Khochbin et al., 2001). As a result, diverse gene expression patterns and physiological outcomes can be regulated by different HDACs and binding partners (Khochbin and Wolffe, 1997). For example, class I HDACs such as RPD3 and HDAC1 make complexes with SIN3 to alter the transcription of their target genes (Pile and Wassarman, 2000). Class II HDACs, instead of binding to SIN3, bind to MEF2 family of transcription factors through MEF2 binding domain in the amino terminal of the enzymes to inhibit MEF2-dependent

Table 1
Classification of histone deacetylases

	Class I	Class II	Class III
Yeast	RPD3 (S22284)	HDAI (P53973) HOS1 (Q12214) HOS2 (X91837) HOS3 (Q02959)	SIR2 (CAA25667)
Drosophila	RPD3 (AF026949) dHDAC3 (AF071559)	dHDAC4 (AE003492) dHDAC6 (AF129433)	dSIR2 (AF068758) dSIRT2 (AE003730) dSIRT4 (AE003435) dSIRT6 (AE003686) dSIRT7 (AE003768)
Human	RPD3 (U31814) HDAC1 (U50079) HDAC3 (U75697) HDAC8 (AF230097)	HDAC4 (AB006626) HDAC5 (AF039691) HDAC6 (AJ011972) HDAC7 (AC004466) HDAC9 (AY032738) HDAC10 (AF407273)	SIRT1 (AF083106) SIRT2 (AF083107) SIRT3 (AF083108) SIRT4 (AF083109) SIRT5 (AF083110) SIRT6 (AF233396) SIRT7 (AF233395)

Figures in parenthesis indicates GenBank accession number.

transcription. Accordingly, class II HDACs play important roles in muscle-specific gene activation, as well as smooth and skeletal muscle differentiation (McKinsey et al., 2001). The role of individual HDACs in regulating gene expression patterns and cellular functions, however, is currently unclear. It would be of great interest to determine the tissue specificity and time-dependent expression of each HDACs. Further mutational studies with HDACs in combination with gene expression profiling are necessary to elucidate the function of each HDAC. These studies will provide a better understanding of the underlying mechanisms of gene regulation that may be important for aging in animals.

3. SIR2

Much of our understanding of the role of HDACs in regulating aging comes from studies using the budding yeast, *S. cerevisiae*, as the model organism. Aging in yeast is marked by both functional and structural changes including increase in cell size, generation time, number of budding scars, sterility, and fragmentation of the nucleolus (Defossez et al., 2001). Yeast cells undergo a limited number of cell divisions and mortality rate increases exponentially as a function of replicative age (Jazwinski et al., 1989). Small budding daughter cells come from the bigger aging mother cells by asymmetric cell division. The daughter cells have normal division potential and do not contain the damages seen for the aging mother cells (Kennedy et al., 1994). SIR2, a NAD^+-dependent HDAC, is thought to regulate cellular senescence in yeast by transcriptional silencing, repression of recombination at the RDN1 locus, and by controlling cellular metabolism.

Transcriptional silencing, a process that leads to transcriptional inactivation of genes located in sections of the chromosome, occurs at the telomeres, HM loci, and the RDN1 locus (Defossez et al., 2001; Jazwinski, 2000), and is associated with replicative aging in yeast. Silencing of subtelomeric regions, for example, decline with increased replicative age in yeast (Kim et al., 1996). Loss of silencing at the HM loci leads to sterility and shortened lifespan by allowing the co-expression of the *a* and α-specific genes (Gottschling et al., 1990). The genes involved in silencing include the silent information regulator (SIR) proteins: SIR2–4. It is thought that the SIR2/3/4 complex is recruited to the nucleus to repress transcription by deacetylating the histones (Defossez et al., 2001). Concordantly, a mutant of SIR4 (SIR4–42), which results in constitutive SIR2/3/4 complex in the nucleus, shows increased lifespan (Kennedy et al., 1997). In telomeric silencing, SIR2 participates by deacetylating histones while SIR3 and SIR4 binds to the hypoacetylated histones to compact chromatin (Galy et al., 2000; Defossez et al., 2001). SIR2 alone also has been shown to cause transcriptional silencing at the RDN1 locus and is discussed in more detail in the following sections.

The RDN1 locus in yeast is composed of a 100–200 repeated copies of the genes encoding ribosomal RNAs. Disruption of SIR2 HDAC abolishes RDN1 silencing (Bryk et al., 1997) and *sir2* mutants exhibit reduced lifespan (Kaeberlein et al., 1999), suggesting that RDN1 silencing by SIR2 is associated with aging. In fact, the level of SIR2 is important for regulating lifespan: an extra copy of *sir2* integrated into the genome results in longer lifespan while deletion of *sir2* causes shortening of lifespan in yeast (Kaeberlein et al., 1999). Intriguingly, overexpression of SIR2 by placing the *sir2* gene under the control of the

galactose-inducible GAL10 promoter in high copy number vector in yeast showed toxicity (Holmes et al., 1997). These results suggest that an optimal amount of SIR2 is needed to increase budding yeast longevity.

The cause of aging associated with RDN1 locus in yeast is hypothesized to be that silenced chromatin reduces homologous recombination between rDNA repeats, and thus, the amount of extrachromosomal ribosomal DNA circles (ERCs) (Sinclair and Guarente, 1997). The link between ERCs and the cause of aging, however, is controversial. Supports for ERCs as the cause of aging come from several studies. First, suppression of RDN1 recombination and ERC formation by deletion of *fob1*, a gene encoding proteins that block rDNA replication, extends lifespan (Defossez et al., 1999). Second, inhibition of ERCs by *fob1* deletion restores the lifespan of *sir2* mutant to normal (Defossez et al., 1999). Third, introduction of artificial ERCs reduce the lifespan of yeast (Sinclair and Guarente, 1997). An equally convincing argument against ERCs as the cause of aging comes from the observation that petite yeast cells have a high level of ERCs, yet they have longer lifespan than their isogenic parent strains (Kirchman et al., 1999). In addition, *sgs1* mutant with defect in the Werners-like DNA helicase exhibits shortened lifespan without an accumulation of ERCs (Heo et al., 1999). Furthermore, although ERC-like extrachromosomal DNA species have been detected in some mammalian cells, there is no evidence of ERC accumulation in aging animals (Johnson et al., 1999). These results suggest that ERC accumulation may not be the universal cause of aging.

SIR2 also regulates longevity by participating in the caloric restriction pathway. Genetic manipulation or physiological changes that provide caloric restriction extend the budding lifespan of yeast. Normally, caloric restriction and extension of lifespan is elicited by reducing the glucose concentration in the growth media from 2 to 0.5% or by disrupting the cyclic AMP-dependent protein kinase A (PKA) pathway, which signals glucose availability in cells (Broach, 1991). These two treatments have been shown to affect the same pathway, since lowering glucose in the growth media of the mutant of PKA pathway did not lead to further extension of lifespan (Lin et al., 2000). Lin et al. have shown that the extension of lifespan by caloric restriction was abolished by the disruption of *sir2* (Lin et al., 2000). It is thought that caloric restriction slows the metabolism to generate more free NAD co-factor required for SIR2 HDAC activity (Guarente and Kenyon, 2000). Consistent with this hypothesis, an increase in NAD resulted in more active SIR2 deacetylase activity (Imai et al., 2000), and caloric restriction extended lifespan and lowered recombination at RDN1 locus (Lin et al., 2000).

Studies using *C. elegans* as a model system also demonstrated that increasing the copy number of yeast homolog of *sir2* (*sir2.1* in *C. elegans*) extended lifespan (Tissenbaum and Guarente, 2001). The *sir2.1* was found to function in the same pathway as *daf16* in the insulin-like signaling pathway, suggesting that *sir2.1* in *C. elegans* may couple nutrient accessibility to longevity, similar to the mechanism found in yeast. *C. elegans* contains four genes that are similar to yeast *sir2*, hence, it is unclear why only *sir2.1* was able to affect lifespan while extra copies of other related *sir2* proteins (*sir2.2*, *sir2.3* and *sir2.4*) had no effect or reduced the lifespan. It will be necessary in the future to determine whether *sir2.1* and other *sir2* related proteins in *C. elegans* contain HDAC activities, and if so, how these proteins differ in their activities. It is possible that other *sir2* related proteins in *C. elegans* also contain HDAC activity, but with different specificity. As a result, different

overall histone acetylation patterns may regulate distinct gene expression, and thus, generate different physiological environments. In addition, it is important to measure the amount of SIR2.1 in animals. The *C. elegans* strain containing the chromosomal duplication including *sir2.1* region does not show significant increase in lifespan. However, transgenic animals carrying unknown copy numbers of the extra genomic fragment containing *sir2.1* or animals having multiple copies of stably integrated extrachromosomal *sir2.1* show significant increase in lifespan. These results, together with the report demonstrating that high copy of *sir2* can cause lethality in yeast, imply that the level of SIR2 expression may be an important factor in regulating longevity. It will also be of interest to investigate the effect of SIR2 on longevity in higher organisms.

Recent reports showed that human SIR2 regulates the p53 tumor suppressor by binding to and deacetylating p53 (Vaziri et al., 2001; Luo et al., 2001). Normally, p53 can be activated by various stresses including DNA damage, oncogenes, and oxidative stress to induce apoptosis or cell cycle arrest. Transgenic mice with elevated p53 activity are highly resistant to tumors, but age prematurely (Tyner et al., 2002). These data suggest that there may be a link between early aging induced by hyperactive p53 and SIR2 expression, since SIR2 overexpression prevents p53 activity and inactivation of SIR2 enhances p53 function. It would be of great interest to determine the lifespan of transgenic mice with SIR2 overexpression and investigate the p53 activity. Transgenic mice with reduced p53 activity can be generated and the lifespan of the mice can be measured. It is likely that extension of lifespan could be accomplished by the proper balance of p53 and SIR2 activities.

4. RPD3

The importance of HDACs in regulating longevity is also demonstrated by RPD3, a class I HDAC. The deletion of yeast *rpd3* increases the budding yeast lifespan and transcriptional silencing at HM, subtelomeric, and RDN1 loci (Kim et al., 1999). Deletion of another HDAC, HDA1, however, had no effect on longevity although an increase in subtelomeric silencing was observed. These results suggest that telomeric silencing alone is not sufficient to cause aging and that silencing at HM and RDN1 loci are important for regulating longevity. It is puzzling, however, that deletion of *rpd3* or *hda1* increased silencing while hyperacetylation of histones was observed (Rundlett et al., 1996). An increase in histone acetylation normally "loosen" the chromatin and decreases transcriptional silencing. Thus, it has been proposed that different acetylation patterns of the core histones determine the intensity of heterochromatic silencing. This is partially supported by data showing that *rpd3* deletion preferentially influenced histone H4 lysine positions 5 and 12, *hda1* deletion affected acetylation at all positions, but to a lesser extent at H4 positions 8 and 16 (Rundlett et al., 1996), and SIR2 can specifically deacetylate lysines 9 and 14 of H3 and lysine 16 of H4 (Imai et al., 2000). Given these different HDAC specificities, different global acetylation patterns may result in different transcriptional regulation.

Another apparent disparity exists between the SIR2 and RPD3 studies. Deletions of both HDACs cause histone hyperacetylation, but *sir2* deletion shortens lifespan while *rpd3* deletion extends lifespan (Kim et al., 1999). We believe that an overall change in gene expression pattern, in addition to gene silencing at the HM, subtelomeric, and RDN1 loci, may mediate

lifespan. Recent gene expression profile studies in yeast showed that *rpd3* deletion caused a two-fold down-regulation of 264 genes and up-regulation of 170 transcripts (Bernstein et al., 2000). The *sir2* deletion, on the other hand, led to a two-fold down-regulation of 10 genes and up-regulation of 57 genes (Bernstein et al., 2000). These results suggest that histone deacetylation by RPD3 and SIR2 can alter the global gene expression pattern by both enhancing and repressing transcription of numerous genes, and not just genes in the telomeric, HM, and RDN1 loci. The genomic studies also showed an interesting difference in the class of genes up-regulated by *sir2* and *rpd3* deletions, suggesting that SIR2 and RPD3 normally affect genes of distinct physiological functions. It will be informative to examine the common sets of genes altered by *sir2* overexpression and *rpd3* deletion, which resulted in extension of lifespan.

A study on the effect of a general HDAC inhibitor, phenylbutyrate (PBA), on the extension of lifespan also supports that alteration of gene expression pattern in animals is important for longevity. A more in depth review of the effect of PBA on lifespan is discussed in the subsequent section. In brief, PBA treatment resulted in histone hyperacetylation, a global change in gene expression pattern, and lifespan extension of *Drosophila*.

5. Inhibitors of histone deacetylases

Structural modifications by acetylation or deacetylation on the amino-termini of histones can regulate the ability of transcription factors and RNA polymerases to bind to DNA for transcription. This process may also determine the specific temporal and spatial gene expression patterns in animals. Thus, it is important to investigate the physiological changes in animals brought upon by alteration in the level of histone acetylation, which can be studied by using inhibitors of HDACs. Recently, HDAC inhibitors have received attention as potential therapeutic drugs for several diseases (Krämer et al., 2001; Marks et al., 2001). HDAC inhibitors inhibit tumor growth and have been used to treat several diseases including urea cycle disorder (Brusilow et al., 1984), tumors (Wang et al., 1999; Melchior et al., 1999), sickle cell anemia (Perrine et al., 1993; Dover et al., 1994; Collins et al., 1995), adrenoleukodystrophy (Kemp et al., 1998), cystic fibrosis (Rubenstein and Zeitlin, 1998), and Fragile X syndrome (Chiurazzi et al., 1999). There are 17 different HDAC inhibitors, ranging from very simple short-chain fatty acid, butyrate, to complicated compound such as depsipeptide. The concentrations necessary to inhibit HDACs in cell cultures and in vivo vary greatly among inhibitors. Furthermore, the specificity of each inhibitor for different HDACs has not yet been demonstrated. In Table 2, we show the structural classification of the known inhibitors of HDACs.

Our group has recently demonstrated that a general HDAC inhibitor, phenylbutyrate (PBA), can extend the lifespan of *Drosophila*. Flies fed with PBA not only exhibited significant extension in both mean and maximum lifespan, but also maintenance of physical vigor (Kang et al., 2002). The range of PBA concentrations effective for increasing lifespan is quite narrow: 10 mM of PBA extends longevity, while higher concentrations are toxic and lower concentrations have no effect. These results are consistent with the level of histone acetylation. Acetylations of histone 3 and 4 increased with increased dosage of PBA. Moreover, PBA increased the lifespan of *Drosophila* regardless of the genetic background.

Table 2
Classification of histone deacetylase inhibitors

Short-chain fatty acids	Hydroxamic acids	Benzamides	Epoxides	Cyclic tetrapeptide containing a 2-amino-8-oxo-9,10-epoxy-decanoyl (AOE) moiety	Cyclic tetrapeptide without an AOE moiety
Butyrate Phenylbutyrate (PBA) Valproic acid	*m*-Carboxy cinnamic acid bishydroxamide (CBHA) Oxamflatin Pyroxamide Scriptaid Suberic bishydroxamic acid Suberoylanilide hydroxamic acid (SAHA) TPX-HA analog (CHAP) Trichostatin A (TSA)	MS-275 *N*-acetyldinaline	Depudecin	Trapoxin	Depsipeptide apicidin

Data in the table are taken from Krämer et al. (2001) and Marks et al. (2001).

Table 3

Function	Gene	Fold-change
Genes induced in PBA-treated flies		
Detoxification	Superoxide dismutase	51.9
	Cytochrome P450-4d1	7.2
	Glutathione *S*-transferase	4.6
Chaperon	*hsc70*	4.5
	hsp60	6.7
	dnaJ like2	2.9
Translation	Translational elongation factor 1α	4.1
Neurotransmitter	Inebriated	26.8
Transcription factor	Daughterless	8.0
Ligand binding	Transportin	8.0
Signal transduction	Epididymal secretory protein	7.5
Transporter	Mitochondrial phophate carrier protein	5.1
Growth factor	Imaginal disc growth factor 1	5.3
Genes repressed in PBA-treated flies		
Metabolism	Glyceraldehyde-3-phosphate dehydrogenase-1	3.7
	NADH: ubiquinone reductase 75 kDa subunit precursor	25.3
	Cytochrome *c* oxidase	6.6
	Peptidyl gycine a hydroxylating monooxygenase	2.1
	Fatty acid synthetase	2.3
	Cytochrome *c* oxidase subunit-VIb	2.7
	Hexokinase	5.0
Chaperon	dnaJ like1	13.2
DNA binding	*osa*	5.0
Ligand binding	Calreticulin	26.5
Signal transduction	Peroxisomal farnesylated protein	4.0
Kinase	Cyclin-dependent kinase-9	2.7
Ion channel	Porin	2.1

Data in the table are taken from Kang et al. (2002).

Caloric restriction is known to extend the lifespan of animals, but the increase in lifespan of *Drosophila* following PBA feeding is not due to caloric restriction. This is evident in the similar weight and size of flies fed with or without PBA. In addition, the number of eggs produced, the percentage of eggs yielding adult progeny, and the weight and size of progeny were the same between flies raised on normal medium and flies fed with PBA, excluding the possibility that lifespan extension is due to changes in reproductive system.

Lifespan extension by PBA is accompanied by hyperacetylation on the tails of H3 and H4, and changes in gene expression. Expression analyses using DNA microarrays indicate that several hundreds of genes are either up- or down-regulated by PBA treatment. Table 3 shows a partial list of genes induced or repressed by PBA treatment as grouped by gene function. Several of the induced genes have previously been shown to enhance longevity: superoxide dismutase (SOD) (Orr and Sohal, 1994; Parkes et al., 1998; Sun and Tower, 1999), elongation factor 1α (Webster and Webster, 1984), glutathione *S*-transferase, cytochrome P450 (Arking, 1998; Mannervik, 1985), and three chaperones (Lithgow, 1996; Tatar et al., 1997). The global change in gene expression pattern and extension of lifespan by PBA support the hypothesis that an overall change in the molecular environment can alter lifespan.

It is important to note that transcription of superoxide dismutase is most dramatically affected by PBA treatment—the SOD transcript level is 50 times higher in flies fed PBA. This may explain why the PBA fed flies are more resistant to stress such as paraquat, a free radical generator. Reactive oxygen species (ROS) are generated by normal cellular activities, and free radicals cause damage in cells and lead to deleterious effects in animals, which may be one of the causes of aging. However, there are defense systems that get rid of ROS, including the enzymes SOD, catalase, glutathione peroxidase, and other antioxidant molecules such as Vitamins C and E (Arking, 1998). Studies have shown that genetic manipulation of SOD affects the lifespan of animals. The lifespan of *Drosophila* lacking Cu/Zn SOD is reduced (Phillips et al., 1989). Human SOD expression in fly motor neurons increases lifespan (Parkes et al., 1998), although expression of catalase in motor neurons has no effect on the lifespan (Phillips et al., 2000). There has been no clear explanation for why SOD expression in motor neurons increases *Drosophila* lifespan. Moreover, the extent of lifespan extension by SOD overexpression varies according to genetic background. Nevertheless, recent work has shown that superoxide dismutase/catalase mimetics such as EUK8 and 134 can extend the lifespan of *C. elegans* by enhancing antioxidant activity in cells (Melov et al., 2000). Treatment with EUK8, 134, and 189 also extended the lifespan of the SOD null mice that normally die within the first week of life (Melov et al., 2001). It would be interesting to examine whether these drugs can extend the lifespan of the normal animal. It is also important to measure the SOD activity in animals fed with the drug.

Another interesting observation with PBA-treated flies is that establishment of an altered cellular environment by PBA, albeit for a limited period, can extend the lifespan of flies, possibly by inhibiting the accumulation of damage, and/or stimulating repair mechanisms (Kang et al., 2002).

There have been various reports of global molecular changes associated with aging, based on studies comparing tissues from young and old animals (Lee et al., 1999; Shelton et al., 1999; Zou et al., 2000). Recently transcriptional changes were measured and compared in aging and paraquat treated *Drosophila*. Many genes were changed by senescence and the free radical generator; however, a number of genes changed by aging were not modified by paraquat, suggesting that other factors are involved in aging in addition to oxidative stress (Zou et al., 2000). Thus, it will be interesting to further investigate the effect of other genes induced or repressed by PBA on the extension of lifespan. In addition, determining whether other HDAC inhibitors can also regulate lifespan of *Drosophila* similar to PBA and the common set of genes altered by different HDAC inhibitors will provide important clues as to the genes responsible for aging.

6. Conclusion

The biology of aging is an intriguing, but complex process. The findings that HDACs can regulate the lifespan of different organisms have shed new light on the underlying mechanism of aging. Overexpression of SIR2 protein in both yeast and *C. elegans* is implicated in silencing of gene transcription and extension of lifespan, whereas deletion of a different HDAC (RPD3) also extends lifespan. However, studies have shown that these two HDACs acetylate different lysine residues located on the N-terminal tails of histones, as well as

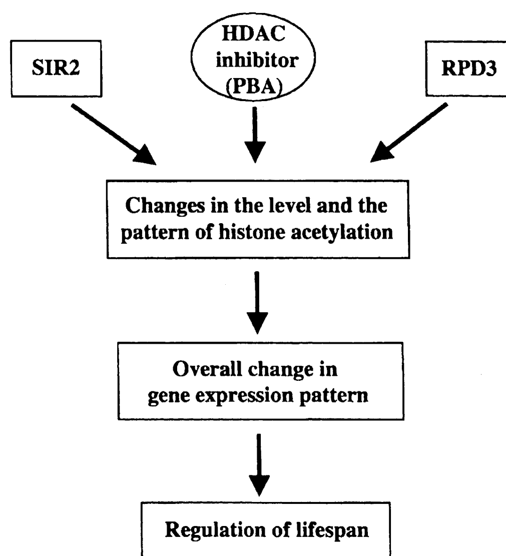

Fig. 2. Model of lifespan extension. Histone deacetylases such as SIR2 and RPD3, as well as HDAC inhibitor, PBA, can induce modification of histones by acetylation or deacetylation. The resulting global change in gene expression can lead to regulation of lifespan.

regulate very different subsets of genes. The underlying mechanism for the extension of lifespan may be that changes in histone acetylation give rise to an optimal balance of expressed and repressed genes to regulate a favorable physiological and cellular environment for longevity (Fig. 2). That would be consistent with the concentration-dependent lifespan extension observed for PBA-treated flies. The genes induced or suppressed by PBA will be of interest in future studies.

It is intriguing that PBA treatment late in the life of the adult fly can still be effective. Our results that simple feeding of a drug can enhance lifespan, along with improved maintenance of vigor, strongly suggests that normal aging can be delayed even late in life. This makes *Drosophila* a convenient model organism for a high throughput screen to identify drugs that can treat premature aging diseases and halt the adverse effects of aging. In addition, transgenic constructs can be readily made in *Drosophila* to test for the effects of overexpression or silencing of individual genes. By identifying the common features of the control regions for the different gene responses, this system may also be useful in understanding the basic mechanisms of histone acetylation and transcriptional regulation of gene expression.

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