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RESEARCH ARTICLE

P/CAF-mediated spermidine acetylation regulates histone acetyltransferase activity

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Abstract

Histones and polyamines are important determinants of the chromatin structure. Histones form the core of nucleosome particles and their modification by acetylation of N-terminal tails is involved in chromatin structural changes and transcriptional regulation. Polyamines, including spermidine, are also targets of both cytoplasmic and nuclear acetylation, which in turn alters their affinity for DNA and nucleosomes. Previous studies report the interplay between polyamines metabolism and levels of histone acetylation, but the molecular basis of this effect is still unclear. In this work, we have analyzed the *in vitro* effect of spermidine on histone H3 acetylation catalyzed by P/CAF, a highly conserved histone acetyltransferase (HAT) (E.C. 2.3.1.48). We have observed that spermidine at very low concentrations activates P/CAF, while it has an inhibitory effect at concentrations higher than 4 μM. In addition, the *in vitro* bimodal effect of spermidine on histone H3 acetylation was also distinctly observed *in vivo* on polytene chromosomes of *Drosophila melanogaster*. We also performed kinetic studies indicating that the activating effect of low spermidine concentrations on P/CAF-HAT activity is based on its involvement as a substrate for P/CAF to produce N^8-acetylspermidine that is able in turn to increase the enzyme activity up to four fold.

Keywords

Histone H3 acetylation, N8-acetylspermidine, P/CAF, spermidine, apermidine acetylation

History

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Introduction

The polyamines are aliphatic cations involved in a large number of cellular processes, including nucleic acid packaging, DNA replication, transcription, translation and apoptosis. These multivalent cations are able to form complexes with negatively charged groups of DNA, RNA and proteins, resulting in chromatin and nuclear enzymes stabilization[1]. Tight regulation of polyamines intracellular pools is fundamental for cell homeostasis; insufficient levels of polyamines result in growth defects and, in some cases, cell death, whereas high polyamines levels can lead to cellular transformation[2]. Cellular polyamine homeostasis comes as a result of their biosynthesis, catabolism and transport processes. The main polyamine catabolic enzyme, spermidine/spermine N^1-acetyltransferase (SSAT or SAT), located into the cytoplasm, is responsible for the excretion of polyamines[3] by providing them as N^1-acetylderivatives to the downstream polyamine oxidase[4]. Specificity and kinetics of rat liver SSAT have already been reported[5]. The over-expression of SSAT, along with the reduction of polyamine pools, has been associated with cell growth inhibition[6].

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In addition to the cytoplasmic polyamine acetyltransferase, mammalian cells also have a nuclear enzyme of unclear function that acetylates the N^8-amino group of spermidine[7]. Acetylation of spermidine to N^8-acetylspermidine was first reported with the identification of a nuclear acetyltransferase, highly related to HATs[8], providing an intriguing link between spermidine metabolism and histone acetylation[8–10]. Interestingly, an inverse relationship between the nuclear spermidine N^8-acetyltransferase and histone acetyltransferase activities was evidenced during the process associated with liver regeneration[11]. Another interesting finding is that APAH, an inhibitor of N^8-acetylspermidine deacetylase, has been reported to induce a significant increase of L1210 cell growth[12]. In terms of polyamine function in the nucleus, several studies have also implicated polyamines in the formation of high order chromatin fibers. Indeed, spermidine has been shown to facilitate condensation of chromatin fragments in *in vitro* experiments[13]. Furthermore, polyamines have also been reported to interact specifically with nucleosome core particle and DNA *in vitro*[14].

Nucleosome folding and oligomerization are dependent on histone N-terminal domains and affected by histone hyperacetylation. P/CAF-HAT is a member of one of the many classes of histone acetyltransferases[15], often associated to transcriptional co-activators or repressors[16,17]. P/CAF activity, targeting preferentially histone H3[17], has been considered important in histone

acetylating events that regulate gene in order to modulate transcription[18].

In the present work, we have explored the potential role of spermidine in histone acetylation by P/CAF-HAT. In particular, we show that spermidine acts as bimodal modulator of histone H3 acetylation in both ''*in vitro*'' and ''*in vivo*'' model systems.

Experimental procedures

Histone H3 acetylation analysis

Recombinant *Drosophila* histones were prepared as previously described[19]. Effects of spermidine (Sigma S4139, Sigma, Chicago, IL) on histone acetylation were monitored incubating in a volume of 20 ml different spermidine concentrations with 3.5 mM recombinant H3, 0.11 mM active PCAF (Millipore, San Francisco, CA, # 14-309) and 5 mM acetylCoA (Sigma, Chicago, IL, # A2056) in HAT buffer (50 mM Tris-HCl pH 8, 10% glycerol, 0.1 mM EDTA, 1 mM dithiothreitol), for 30 min at 30 °C. Histone H3 acetylation was analyzed by Western blotting analysis with antibody against acetylated histone H3 (Upstate, Greenville, SC, # 06-599, recognizing histone H3 acetylations on K9 and K14). Acetyl-histone H3 (lys9-lys14) peptide (# 12-360) was supplied by Upstate (Greenville, SC). The chemiluminescent HRP-conjugated secondary antibody was developed using the Super Signal West Femto substrate (Pierce, Waltham, MA) and acquired with the ChemiDoc XRS imager (BioRad, Hercules, CA). Densitometric analysis of bands was performed with QuantityOne software (Biorad, Hercules, CA).

Spermidine acetylation analysis

Spermidine acetylation was monitored incubating in a volume of 200 µl spermidine (0.5–40 µM) 1 µg of active PCAF (Millipore, San Francisco, CA, # 14-309) and 2.5, 5 or 10 µM acetyl-CoA (Sigma, Chicago, IL, # A2056) in 50 mM Tris-HCl pH 8.0, for 30 min at 30 °C. Polyamine analysis was performed using a modification of our previous RP-HPLC procedure[20]. Briefly, incubated samples were treated as previously reported and, after derivatization, the dryness samples were dissolved in 20 µl of 50% methanol, 2 µl aliquots were injected into the HPLC system and separation was performed using a gradient of solvent A (water) and B (methanol). Polyamines were eluted from 52% B (0–2 min) to 58% B (8–14 min) at room temperature. The gradient was then immediately returned to 53% solvent B (14–15 min) and the initial conditions restored in 4 min. The flow rate was 0.25 ml/min and polyamines detected at 229 nm and 254 nm (detector sensitivity 0.01 a.u.f.s.).

Bi-substrate kinetic measurements of P/CAF-SAT activity

The bi-substrate kinetic analysis was performed at acetyl-CoA concentrations spanning from 2.5 to 10 µM and at spermidine concentrations spanning from 0.5 to 10 µM. The P/CAF-SAT activity was monitored via the chromatographic assay previously described. Data obtained at un-saturating acetyl-CoA concentrations (2.5 and 5 µM) were plotted and the curves drawn using the spline method. After determination of the correct position of the asymptote, using the procedure described by Cleland[21], data at 2.5, 5 and 10 µM acetyl-CoA were fitted to the sequential (ternary complex) mechanism equation (1)[22], by the computer program Grafit version 7[23], using a non-linear least-square approach. Data were plotted in double reciprocal form and kinetic constants were determined:

$$v = Vmax[A][B]/(KiaK\mathrm{mB} + K\mathrm{mA}[B] + K\mathrm{mB}[A] +]A][B]) \quad (1)$$

At un-saturating concentration of acetyl-CoA, the kinetic mechanism (substrate inhibition in an ordered bi-reactant system with formation of a dead-end complex) is expressed by equation (2)[22] where *Ki* is the dissociation constant of EB:

$$v = V\mathrm{max}[B]/K\mathrm{mB}(1 + Kia/[A]) + [B](1 + KiaK\mathrm{mB}/Ki[A] + K\mathrm{mA}[A] + K\mathrm{mA}[B]/Ki[A]) \quad (2)$$

In this system, the plot will be linear only at low values of [B], and the minimum occurs at

$$[B]\mathrm{min} = [K\mathrm{mB}Ki([A] + Kia)/K\mathrm{mA}]^{1/2} \quad (3)$$

In these equations, *K*mA is the *K*m value for acetyl-CoA, *K*mB is the *K*m value for spermidine and *K*ia is the dissociation constant for acetyl-CoA. Also, the dissociation constant for B, *K*ib, is determined from the relationship *K*ib = *K*ia (*K*mB/*K*mA). In addition, by equation (3)[22], the dissociation constant of P/CAF-spermidine complex, *Ki* was determined.

P/CAF kinetic measurement with the effectors spermidine and N^8-acetylspermidine

In steady-state activation and inhibition analysis, data are fitted to equation (4)[22] for ordered bi–bi systems with alternative B (spermidine) and to equation (5)[22] for the *V* systems where N^8-acetylspermidine is the non-essential activator. A non-linear least-square analysis was used[23]:

$$v = V\mathrm{max}[B]/K\mathrm{mB}(\mathbf{1} + Kia/[A]) + [I]/K\mathrm{mI} + Kia[I]/K\mathrm{ii}[A]) + [B](1 + K\mathrm{mA}/[A]) \quad (4)$$

$$v/V\mathrm{max} = \alpha(1 + \mathrm{gL})/(1 + \alpha)(1 + \mathrm{L}) \quad (5)$$

Kinetic data processing

Leatherbarrow Eritacus Software made a provisional estimate of kinetic constants with the aid of the Grafit version 7 program[23]. The appropriate velocity equations describing the kinetic behaviors were verified[22].

Analysis of polytene chromosomes

Df[yw] polytene chromosomes were prepared from third-instar larvae grown at 18 °C. After dissection, salivary glands were incubated in Grace's Insect Medium (Invitrogen, Waltham, MA) added with spermidine at different concentrations for 2 h at room temperature. Then polytene chromosomes were processed as described previously[24], using the same primary antibody of Western blot analysis (Upstate, Greenville, SC, # 06-599). DNA was stained with DAPI.

Results

Optimization of *in vitro* histone acetyltransferase (HAT) assay conditions to analyze histone H3 acetylation

In order to analyze spermidine effects on histone H3 acetylation catalyzed by P/CAF, we tested experimental conditions to optimize reaction specificity. We tested a broad concentration range of acetyl-CoA (1–160 µM) on a fixed histone H3 amount (3.5 µM) and observed that high acetyl-CoA concentrations (20 µM or higher) promote a relevant spontaneous acetylation of H3, thus masking the P/CAF specific acetylation (Figure 1A). Then, using acetyl-CoA concentrations yielding the highest enzymatic H3 acetylation and the lowest aspecific acetylation (5–10 µM), the optimal histone H3 concentration was determined as 3.5 µM (Figure 1B).

Spermidine has a bimodal effect on histone H3 acetylation, depending on its concentration

In order to dissect the molecular mechanism inducing histone hypoacetylation following spermidine addition[25], we performed *in vitro* HAT assay to monitor the effect of spermidine on H3 acetylation catalyzed by P/CAF. We observed that the addition of spermidine in the broad concentration range from 0.5 μM to 100 μM induced histone H3 hyperacetylation up to 3 μM concentration, whereas it leads H3 hypoacetylation at 5 μM or higher concentration (Figure 2A and B).

To better determine the spermidine concentration that is critical to switch from activating to inhibitory effects, we performed an *in vitro* HAT assay in presence of spermidine in the concentration range from 0.25 μM to 16 μM. Under these experimental conditions, spermidine showed a peak of activating effect on P/CAF-HAT-dependent H3 acetylation at 4 μM, while we observed an increasing inhibitory effect at higher concentrations (Figure 2C and D). The curve in Figure 2(D) shows the data in Figure 2(C), analyzed using the spline method that produces a smooth curve through the data points.

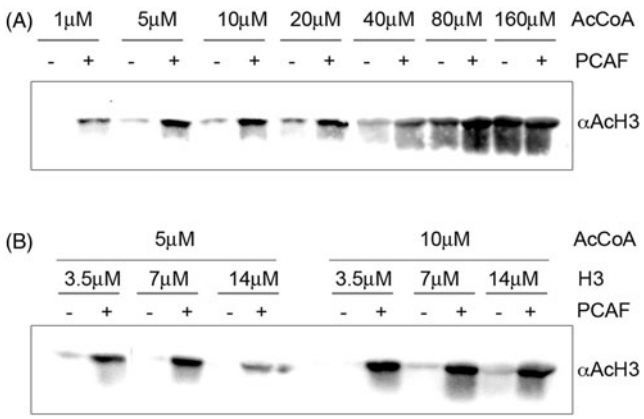

Figure 1. Optimization of PCAF-HAT assay conditions of histone acetylation. (A) Assays of enzymatic and non-enzymatic acetylation reactions of H3 histone were performed at variable acetyl-CoA concentrations with (+) or without (−) P/CAF. The immunoblotting with antibody recognizing AcH3 shows that the production of the acetylated histone H3 (AcH3) is actively catalyzed already at 5–10 μM acetyl-CoA concentration. At higher acetyl-CoA concentration a non-enzymatic acetylation event is apparent. (B) Different H3 concentrations were tested in the presence of a fixed acetyl-CoA amount. Western blotting analysis shows that at 5 μM acetyl-CoA concentration all the different H3 amounts tested gave negligible non-enzymatic acetylation.

P/CAF catalyzes the acetylation of spermidine

In order to better understand the bimodal effect of spermidine on H3 acetylation, we assumed the hypothesis that spermidine could represent a P/CAF substrate[11]. In this respect, we assessed possible spermidine acetylation catalyzed by P/CAF using a chromatographic approach that measures the N-acetyl derivatives of spermidine. The chromatographic profiles, in Figure 3(A), show the N^8-acetylspermidine formation when P/CAF (0.11 μM) was incubated with 5–10 μM spermidine and 5 μM acetyl-CoA. These data clearly show that spermidine acts as a substrate for the enzyme that acetylates it at N^8 position. We also determined the activity of P/CAF spermidine acetyltransferase (P/CAF-SAT) at fixed concentrations of acetyl-CoA (2.5, 5 and 10 μM) by increasing spermidine concentration (from 0.25 μM up to 40 μM) in the presence of 0.11 μM P/CAF. As shown in Figure 3(B), at un-saturating acetyl-CoA concentrations (2.5 and 5 μM), as the plots approach to the 1/*v*-axis, they pass through a minimum and curve up. This kinetic behavior is in agreement with the substrate inhibition in an ordered bi-reactant system where spermidine reacts with P/CAF as well as with P/CAF-acetyl-CoA, in a steady-state system, with formation of a dead-end complex (P/CAF-spermidine)[22] (Figure 3C). To determine the correct position of the asymptotes, the procedure described by Cleland was used[21]. In Figure 3(D) and (E), the 1/*v*-axis intercept and $slope_{1/[B]}$ replots versus 1/[acetyl-CoA] are shown. The average values from two separate experiments yielded a k_{cat} value of $1.8 \times 10^6 \pm 0.09\ min^{-1}$. The *Kia* and *K*mA values for acetyl-CoA were 20 ± 3.6 μM and 1.74 ± 0.22 μM, respectively. The *Kib* and *K*mB values for spermidine were 26.32 ± 4.8 μM and 2.29 ± 0.41 μM, respectively. The *ki* dissociation constant of P/CAF-spermidine complex was 1.51 ± 0.18 μM.

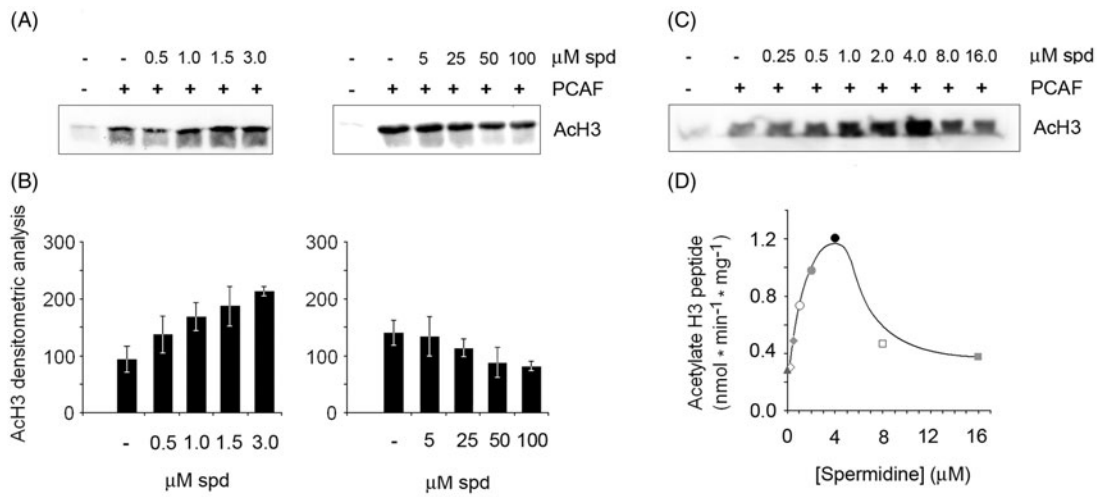

Figure 2. PCAF-HAT activity on histone is inhibited by spermidine at relatively high concentration and activated at low concentration. In (A), Western blotting analysis of H3 acetylation by PCAF in the presence of different spermidine concentrations. Acetyl-CoA and H3 were used at, respectively, 5 μM and 3.5 μM concentration. In (B), quantification of the spermidine effects on PCAF dependent H3 acetylation revealed by densitometric analysis of Western blotting bands. In (C), Western blotting analysis of histone H3 acetylation by PCAF in the presence of spermidine in the concentration range of 0.25–16 μM. In (D), quantification of the spermidine effects on PCAF-dependent H3 acetylation, revealed by densitometric analysis of Western blotting bands, is reported. The curve was drawn using the spline method. Data represent means ± SD from three independent experiments.

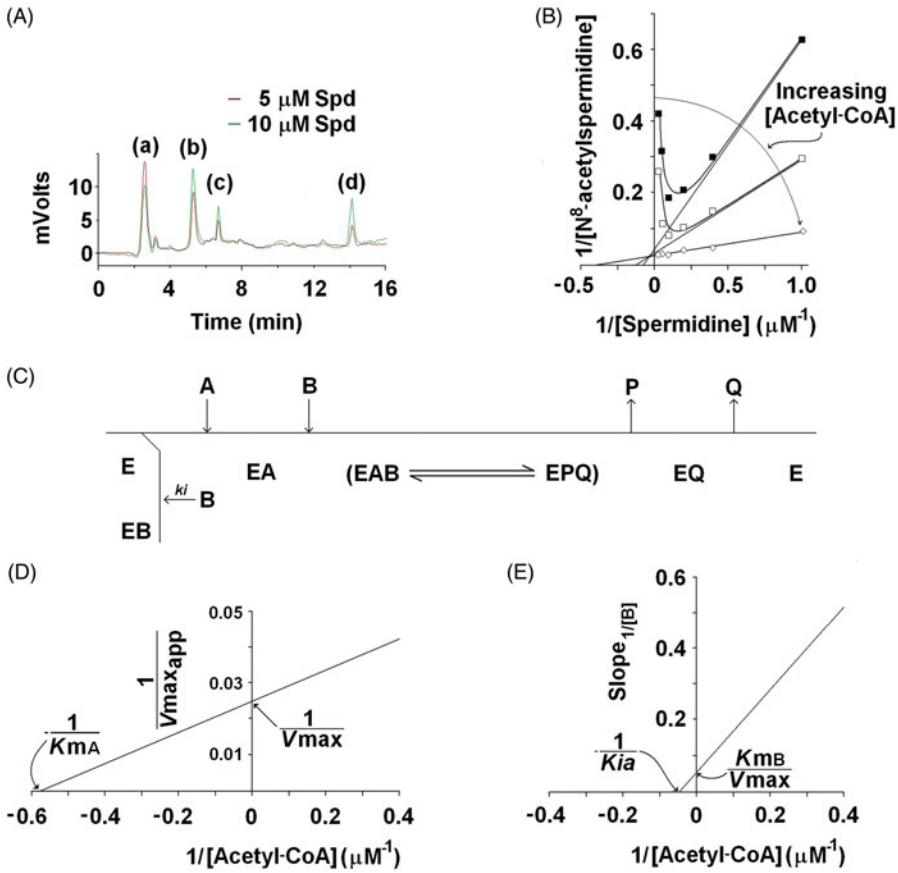

Figure 3. Dual behavior of spermidine: substrate and inhibitor of P/CAF. In (A), chromatographic profiles show benzoic acid (a), N8-acetylspermidine (b) produced incubating spermidine 5 and 10 μM at fixed unsaturating acetyl-CoA concentration (5 μM), methyl benzoate (c), and spermidine (d). In (B), plot of 1/*v* versus 1/[Spermidine] spanning from 1 μM to 40 μM, P/CAF 0.11 μM, and acetyl-CoA 2.5 μM (filled squares), 5 μM (open squares), and 10 μM (open diamonds). Two additional concentrations of spermidine were used (0.25 μM and 0.5 μM), but not displayed. Data were fitted to Equation (1) for a sequential (ternary complex) mechanism (see Experimental procedures) using a non-linear least-square approach. The experiment was performed in duplicate with a representative plot displayed. The substrate inhibition scheme of an ordered bi-reactant system with a dead-end EB complex formation (P/CAF-spermidine) is shown in C. In D and E, 1/v-axis intercept, and Slope1/B replots, for KmA and Kia calculation are shown.

Regulation of P/CAF-HAT activity by N^8-acetylspermidine: kinetic analysis

Assuming that the increment of P/CAF-HAT activity is not due to spermidine but to N^8-acetylspermidine, we evaluated its effect on P/CAF-HAT activity measuring initial rates, without (control) and with 0.05–0.8 μM of N^8-acetylspermidine, holding constant concentrations of acetyl-CoA and histone H3 at 5 and 10 μM, respectively. As shown in Figure 4(A), data plotted as *v* versus [N^8-acetylspermidine], that represents enzyme activator, show sigmoid trend. Considering that P/CAF is, however, active in the absence of N^8-acetylspermidine, it is possible to state that it is a non-essential activator. So, to better evaluate its effect on the kinetic behavior of P/CAF on histone H3 acetylation, we evaluated the initial rates, in absence (control) and in the presence of N^8-acetylspermidine (0.05–0.8 μM), holding constant acetyl-CoA (5 μM) while histone H3 was varied. In Figure 4(B), the data plotted in double-reciprocal form display a line(s) pattern, intersecting the negative share of 1/[S] axis. N^8-acetylspermidine showed an activating effect on P/CAF-HAT as evidenced by the decrease of the line's slope with respect to the control, when its concentration was increased.

Studies on inhibitory effects of spermidine on P/CAF-HAT activity

To evaluate the inhibitory effects of spermidine on P/CAF-HAT, we evaluated the initial enzymatic rates, without (control) and with of 25–100 μM spermidine, holding constant acetyl-CoA (5 μM), while H3 was varied. The data plotted in double-reciprocal form display a classical pattern, describing inhibitory effects (Figure 5A) attributable to spermidine as alternative substrate [EAI] and as dead-end inhibitor [EI] in an ordered bi–bi system (Figure 5B).

Spermidine administration modulates histone H3 acetylation *in vivo*

In order to test the relevance *in vivo* of the histone H3 acetylation control by specific spermidine concentration, already showed *in vitro*, we analyzed the trend of histone H3 acetylation in polytene chromosomes of *Drosophila melanogaster*. After incubation of dissected larval salivary glands in Grace's Insect Medium added with different spermidine concentration, H3 acetylation was analyzed by indirect immune-fluorescence. As shown in Figure 6, treatment of salivary glands with 3 μM spermidine clearly induces histone H3 hyperacetylation, chargeable to N^8-acetylspermidine produced by the SAT activity of P/CAF, whereas incubation of salivary glands with 100 μM spermidine causes a net reduction of histone H3 acetylation when compared to control glands, according to our performed ''*in vitro*'' analysis (Figure 5).

Discussion

Chromatin structure plays a crucial role in the regulation of eukaryotic nuclear reactions, most of which require chromatin modification to work efficiently. These changes on chromatin structure occur as the result of nucleosome remodeling enzymes that work in concert with covalent modifiers of histone tails[26]. In particular, histone acetylation is considered as a major post-translational modification playing an important role in biological processes such as transcription, replication and DNA repair[27]. The balance between histones and acetylated-histones, in the nucleus, is controlled by the opposite activities of histone acetyltransferases (HATs) and histones deacetylases (HDACs) and the precise maintenance of this balance is required for both cellular homeostasis and preventing cell cycle abnormalities and malignancy development[28].

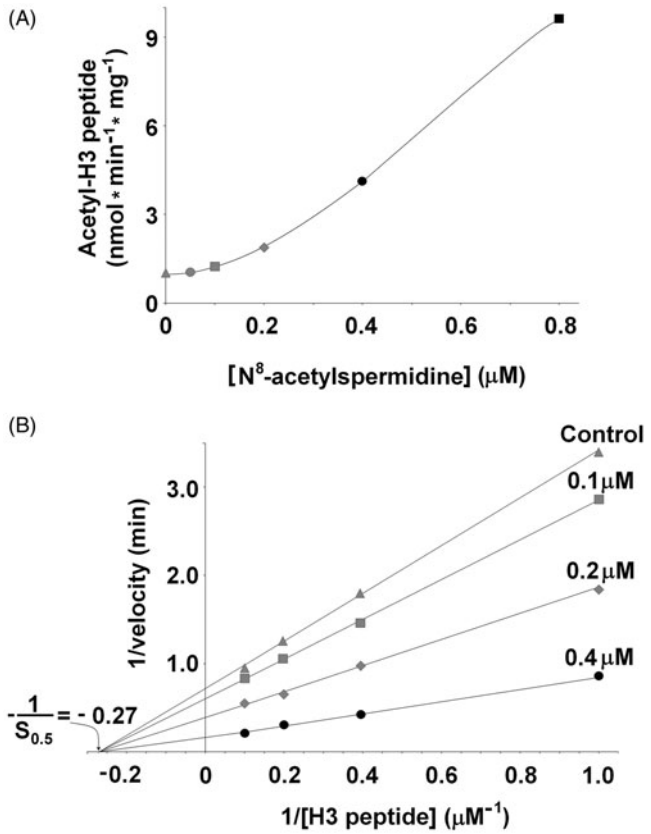

Figure 4. P/CAF-HAT activity: regulation by N8-acetylspermidine. In (A), the sigmoidal curve describes the activity of P/CAF-HAT as a function of N8-acetylspermidine (0.05–0.8 μM), at fixed concentrations of acetyl-CoA (5 μM) and histone H3 (10 μM). In (B), kinetic data of P/CAF-HAT activity relative to the assay performed incubating histone H3 (1–10 μM) and acetyl-CoA (5 μM) in absence (gray triangle) and in presence of fixed N8-acetylspermidine concentration (gray squares, 0.1 μM; gray diamonds, 0.2 μM; filled circles, 0.4 μM). The experiments were performed in triplicate, data elaborated using a non-linear least-square approach, and one of the double reciprocal plots displayed. In this graph, velocity data obtained at 0.05 and 0.8 μM of N8-acetylspermidine are not displayed.

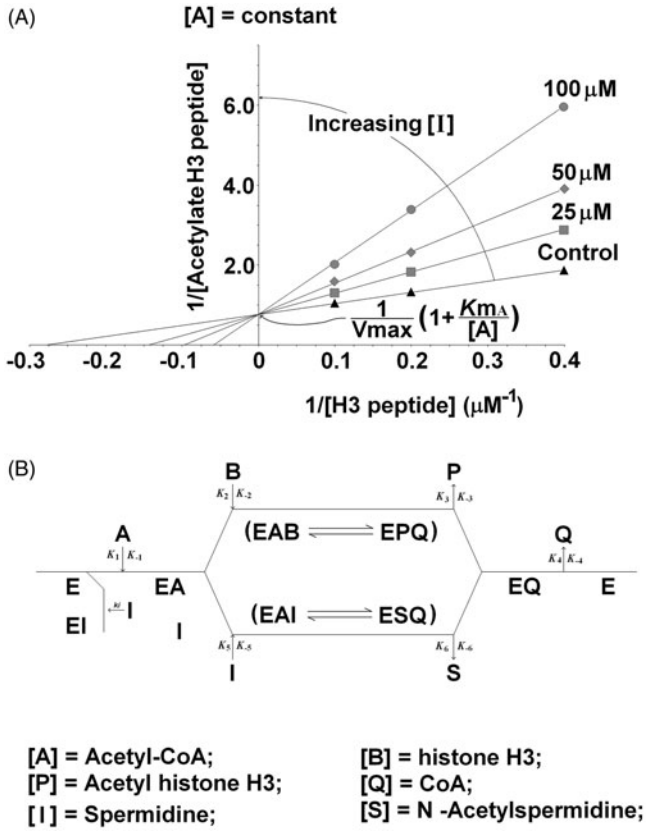

Figure 5. P/CAF-HAT activity: inhibition by spermidine. In (A), the plot in double-reciprocal form of enzymatic rate data related to the inhibitory effect on P/CAF-HAT of spermidine in the concentration range 2.5–100 μM, is shown. Samples containing 0.11 μM P/CAF, 5 μM acetyl-CoA, and 2.5–10 μM histone H3 were incubated in 50 mM Tris-HCl pH 8.0 at 30 °C for 30 min. The experiments were performed in triplicate and data elaborated using a non-linear least-square approach. In (B), scheme relative to the two different inhibition mechanisms of spermidine on P/CAF-HAT, operating as alternative substrate or dead-end inhibitor, is reported.

Polyamines have a multitude of functions affecting growth and development. Several studies have demonstrated that polyamines are involved in gene transcription, modulation of protein kinases, control of ion channel activities and cell cycle[29–35]. Equal to polycations, polyamines are involved in chromatin stabilization and binding to specific nucleosome location, especially at ''TATA'' elements on DNA[1].

The functional interaction between histone acetylation processes and polyamines has been studied in relation to oligomerization of nucleosomal arrays. Reconstituted nucleosomal arrays with intact hypo-acetylated histone octamers fully oligomerize at 200 μM spermidine, whereas 50% more spermidine is required to condense hyper-acetylated nucleosomal arrays[16]. Therefore, the contribution of polyamines to transcriptional regulation *in vivo* may also be controlled by balancing histone acetylation and deacetylation reactions. Acetylation of polyamines reduces their affinity for DNA and nucleosomes and it is thought that polyamines acetylation and histones acetylation act synergistically to modulate chromatin structure[36].

The relationship between polyamines and histone *in vivo* has been considered in various models of ageing through epigenetic modifications, induction of autophagy and suppression of necrosis. Mechanisms responsible for the cytoprotective autophagy induced by spermidine have been partially elucidated in yeast. In this model, spermidine inhibits the activity of HATs, causing histone H3 hypoacetylation, which in turn epigenetically affects gene function, allowing the induction of autophagy relevant transcripts[25].

Our data highlight the inhibitory effect of spermidine on P/CAF HAT activity *in vitro,* with the consequent histone H3 hypoacetylation, confirming previous observation *in vivo*[25]. In particular, we have observed that the inhibitory effect of spermidine on P/CAF activity is dependent on its concentration: we observed in fact a substantial activation of H3 acetylation at spermidine concentrations lower than 5 μM.

Previous studies have reported that the interferon regulatory factor 2 (IRF2) has an inhibitory effect on p300 and P/CAF histone-acetylating activities through its action as a competitive substrate[37]. Likewise, our data suggest that the inhibitory effect of spermidine on histone acetylation at relatively high concentrations (>5 μM) could be ascribed to the behavior of this polyamine as competitive substrate for the P/CAF active site both *in vitro* and *in vivo*.

It is known that spermidine can be acetylated to N^8-acetylspermidine and that this modification is important for polyamine catabolism as it induces the removal of spermidine from cell nucleus[38]. Nevertheless, there is evidence supporting a role of N^8-acetylspermidine itself in the regulation of cell growth and differentiation. Accumulation of N^8-acetylspermidine in L1210 cell cultures results in stimulation of their growth[12], whereas increased N^8-acetylspermidine levels in PC12 cells have

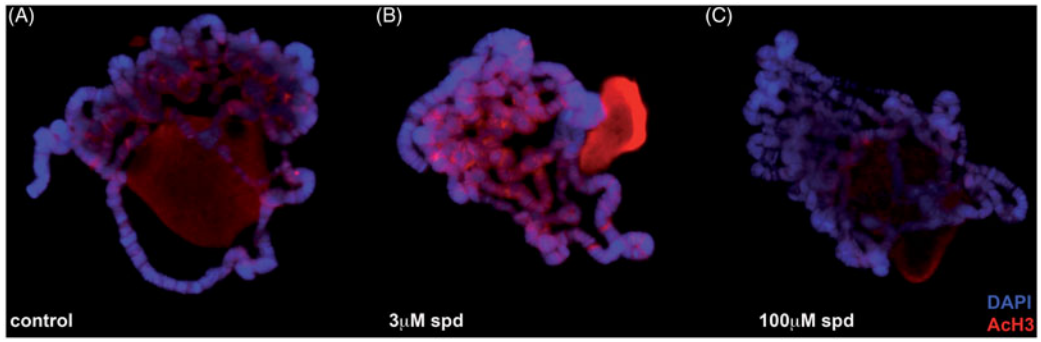

Figure 6. In ''*in vivo*'' effects of spermidine on H3 acetylation\r\nImmuno-fluorescence analysis of *Drosophila melanogaster* polytene chromosomes: control (A) and after treatment with 3 μM (B) and 100 μM (C) spermidine.\r\n.

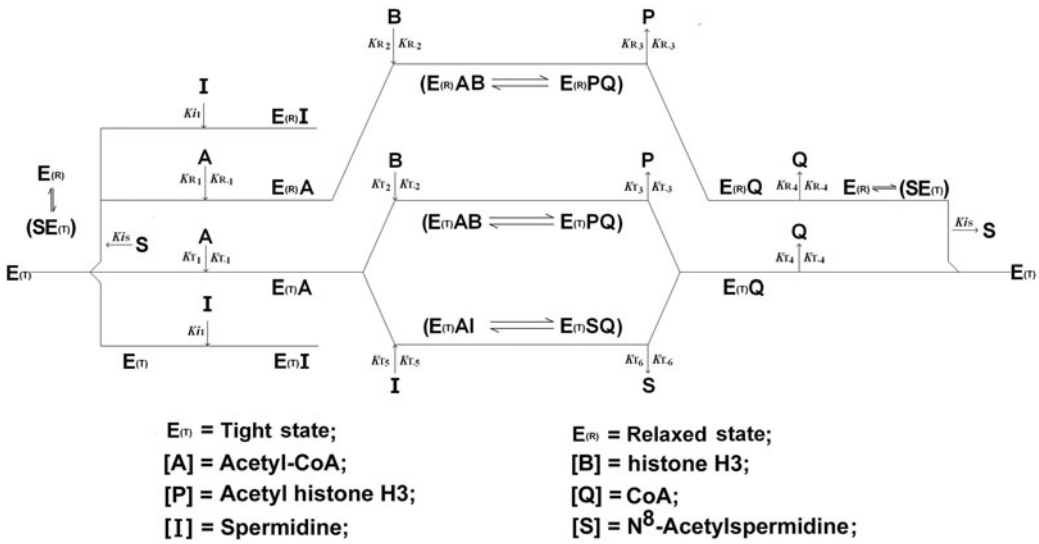

Figure 7. Steady-state kinetics of a bi-reactant enzyme: regulatory role of spermidine and N8-acetylspermidine on P/CAF-HAT and -SAT activities. In the reaction scheme shown below, I (spermidine) is substrate of the enzyme E(T) that leads to the production of S (N8-acetylspermidine) behaving like an alternative substrate for enzyme E(T) which substitute B (histone H3) in the ordered bi–bi mechanism. The product S can bind to E(T), at non-catalytic site, by activating the enzyme which will change into E(R). When the concentration of I is high, it can substitute for A (acetyl-CoA) in the binding either with E(T) or E(R), acting as dead-end inhibitor.

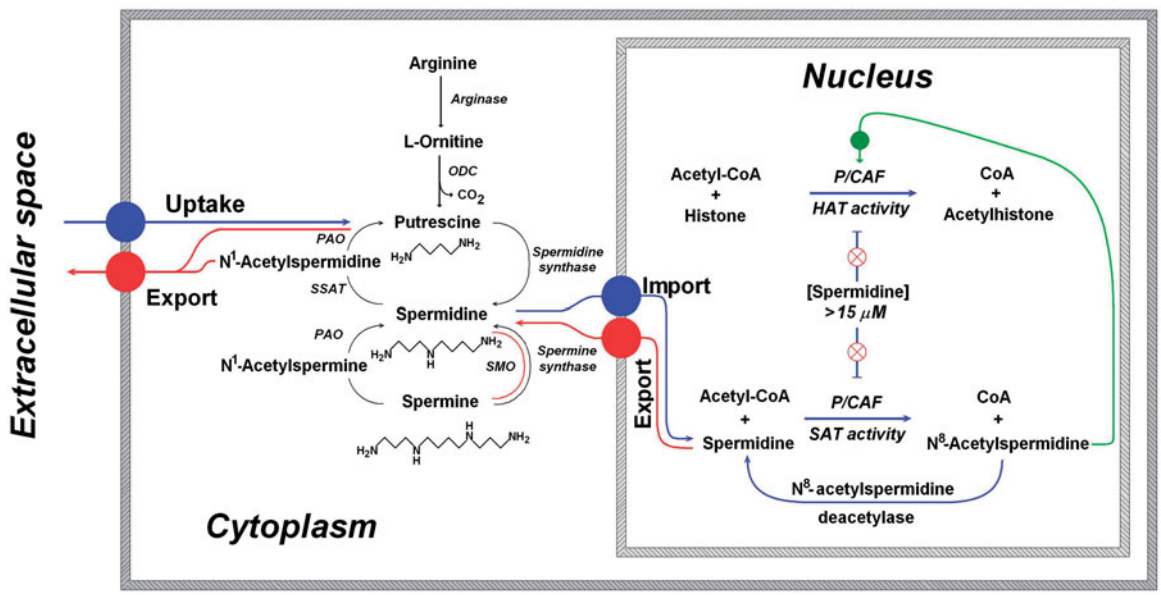

Figure 8. Metabolism of spermidine and its N-acetylderivatives: schematic representation. In cytosol the well-known metabolism of polyamines is reported. In the nucleus, the relationship between spermidine and P/CAF is shown highlighting the role and the effects of this polyamine on the enzyme. Spermidine at concentrations higher than 15 μM exerts a marked inhibitory effect on P/CAF acetylating activity versus histone H3. On the contrary, at lower concentrations (<5 μM) it plays a net increase of the P/CAF-HAT activity. This activating effect of spermidine at low concentrations is explained by the evidence of its conversion to N8-acetylspermidine, catalyzed by P/CAF (P/CAF-SAT activity). Thus, in the nucleus, the acetylation of spermidine will result in the decrease of the inhibitor (spermidine), and in the availability of the activator N8-acetylspermidine.

been associated with the inhibition of growth and differentiation[39]. In this respect, both nuclear localization and substrate specificity suggest that spermidine N^8-acetyltrasferase can be considered as a histone acetylase[11].

We have hypothesized that P/CAF has a role in spermidine acetylation, specifically at N^8-position. Here we report for the first time that P/CAF is implicated in the biosynthesis of N^8-acetylspermidine through an alternative spermidine acetyltransferase activity (P/CAF-SAT) (Figure 7). Considering that P/CAF contains a bromodomain, consisting of amino acid residues 745–815, that is known to interact with acetyl peptides derived from N-terminal H3 and H4 histones[40] and also that GNC5 bromodomain displays a strong preference for positively charged ligands over negatively charged ones[41], we speculate here that the seeming activating effect of spermidine on H3 acetylation, observed at lower concentration (<5 μM), is likely due to binding of N^8-acetylspermidine to the P/CAF bromodomain and the resulting histone acetylating activity. In particular, we suggest that P/CAF is present in two different forms (T and R states) and that binding of N^8-acetylspermidine to its bromodomain produces the enzyme conversion from a less active T state to the more active R state. At higher concentrations (>5 μM), spermidine competes with histone H3 as an alternative substrate, triggering the inhibition of P/CAF-HAT activity (Figure 7). Furthermore, the inhibitory effect of spermidine on P/CAF-HAT at very high concentration (>15 μM) is based on a distinct mechanism, as indicated by kinetic analysis in *in vitro* and direct evaluation of the activity in *in vivo* (Figure 6). Under these conditions, spermidine interacts with P/CAF at the catalytic site for acetyl-CoA, eventually leading to the production of a P/CAF-spermidine dead-end complex (Figure 3C and 7).

Our data provide new insights on the role of polyamines on nucleosome modification, supporting the regulation by spermidine of P/CAF activity, on one hand, and the conversion of spermidine into N^8-acetylspermidine by P/CAF, on the other hand (illustrated in Figure 8). Further studies are required to better define the complex interplay between histones and polyamines in the epigenetic regulation of gene transcription during cell proliferation and differentiation processes.

Declaration of interest

The authors report that they have no conflicts of interest.

References

1. Bjelakovic G, Stojanovic I, Jevtovic Stoimenov T, et al. Metabolic correlations of glucocorticoids and polyamines in inflammation and apoptosis. Amino Acids 2010;39:29–43.
2. Childs AC, Mehta DJ, Gerner EW. Polyamine-dependent gene expression. Cell Mol Life Sci 2003;60:1394–406.
3. Seiler N, Bolkenius FN, Knodgen B. The influence of catabolic reactions on polyamine excretion. Biochem J 1985;225:219–26.
4. Casero Jr RA, Pegg AE. Spermidine/spermine N1-acetyltransferase-the turning point in polyamine metabolism. FASEB J 1993;7:653–61.
5. Della Ragione F, Pegg AE. Studies of the specificity and kinetics of rat liver spermidine/spermine N1-acetyltransferase. Biochem J 1983;213:701–6.
6. Vujcic S, Halmekyto M, Diegelman P, et al. Effects of conditional overexpression of spermidine/spermine N1-acetyltransferase on polyamine pool dynamics, cell growth, and sensitivity to polyamine analogs. J Biol Chem 2000;275:38319–28.
7. Desiderio MA, Mattei S, Biondi G, Colombo MP. Cytosolic and nuclear spermidine acetyltransferases in growing NIH 3T3 fibroblasts stimulated with serum or polyamines: relationship to polyamine-biosynthetic decarboxylases and histone acetyltransferase. Biochem J 1993;293:475–9.
8. Morgan JE, Blankenship JW, Matthews HR. Polyamines and acetylpolyamines increase the stability and alter the conformation of nucleosome core particles. Biochemistry 1987;26:3643–9.
9. Blankenship J, Walle T. Acetylation of spermidine and spermine by rat liver and kidney chromatin. Arch Biochem Biophys 1977;179:235–42.
10. Libby PR. Calf liver nuclear N-acetyltransferases. Purification and properties of two enzymes with both spermidine acetyltransferase and histone acetyltransferase activities. J Biol Chem 1978;253:233–7.
11. Desiderio MA. Opposite responses of nuclear spermidine N8-acetyltransferase and histone acetyltransferase activities to regenerative stimuli in rat liver. Hepatology 1992;15:928–33.
12. Wang Z, Fries D, Blankenship J. Effect of N8-acetylspermidine deacetylase inhibition on the growth of L1210 cells. Biochem Pharmacol 1999;57:1095–103.
13. Bertin A, Mangenot S, Renouard M, et al. Structure and phase diagram of nucleosome core particles aggregated by multivalent cations. Biophys J 2007;93:3652–63.
14. Pollard KJ, Samuels ML, Crowley KA, et al. Functional interaction between GCN5 and polyamines: a new role for core histone acetylation. EMBO J 1999;18:5622–33.
15. Roth SY, Denu JM, Allis CD. Histone acetyltransferases. Annu Rev Biochem 2001;70:81–120.
16. Bannister AJ, Kouzarides T. The CBP co-activator is a histone acetyltransferase. Nature 1996;384:641–3.
17. Ogryzko VV, Schiltz RL, Russanova V, et al. The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell 1996;87:953–9.
18. Schiltz RL, Mizzen CA, Vassilev A, et al. Overlapping but distinct patterns of histone acetylation by the human coactivators p300 and PCAF within nucleosomal substrates. J Biol Chem 1999;274:1189–92.
19. Luger K, Rechsteiner TJ, Richmond TJ. Preparation of nucleosome core particle from recombinant histones. Meth Enzymol 1999;304:3–19.
20. Taibi G, Schiavo MR, Gueli MC, et al. Rapid and simultaneous high-performance liquid chromatography assay of polyamines and monoacetylpolyamines in biological specimens. J Chromatogr B Biomed Sci Appl 2000;745:431–7.
21. Cleland WW. Determining the chemical mechanisms of enzyme-catalyzed reactions by kinetic studies. Adv Enzymol Relat Areas Mol Biol 1977;45:273–387.
22. Segel IH. In: Enzyme kinetics, behaviour and analysis of rapid equilibrium and steady-state enzyme systems. New York: John Wiley & Sons, Wiley Interscience Press; 1975.
23. Leatherbarrow RJ. GraFit Version 7. Horley, UK: Erithacus Software Ltd; 2010.
24. Burgio G, Cipressa F, Ingrassia AM, et al. The histone deacetylase Rpd3 regulates the heterochromatin structure of Drosophila telomeres. J Cell Sci 2011;124:2041–8.
25. Eisenberg T, Knauer H, Schauer A, et al. Induction of autophagy by spermidine promotes longevity. Nat Cell Biol 2009;11:1305–14.
26. Clapier CR, Cairns BR. The biology of chromatin remodeling complexes. Annu Rev Biochem 2009;78:273–304.
27. Kouzarides T. Chromatin modifications and their function. Cell 2007;128:693–705.
28. Selvi RB, Kundu TK. Reversible acetylation of chromatin: implication in regulation of gene expression, disease and therapeutics. Biotechnol J 2009;4:375–90.
29. Gerner EW, Meyskens Jr. FL Polyamines and cancer: old molecules, new understanding. Nat Rev Cancer 2004;4:781–92.
30. Igarashi K, Kashiwagi K. Modulation of cellular function by polyamines. Int J Biochem Cell Biol 2010;42:39–51.
31. Pegg AE. Mammalian polyamine metabolism and function. IUBMB Life 2009;61:880–94.
32. Pegg AE, Casero Jr. RA Current status of the polyamine research field. Methods Mol Biol 2011;720:3–35.
33. Nowotarski SL, Woster PM, Casero Jr. RA Polyamines and cancer: implications for chemotherapy and chemoprevention. Expert Rev Mol Med 2013;15:e3. DOI: http://dx.doi.org/10.1017/erm.2013.3.
34. Battaglia V, De Stefano Shields C, Murray-Stewart T, Casero Jr. RA Polyamine catabolism in carcinogenesis: potential targets for chemotherapy and chemoprevention. Amino Acids 2014;46:511–19.

35. Pietrocola F, Lachkar S, Enot DP, et al. Spermidine induces autophagy by inhibiting the acetyltransferase EP300. Cell Death Differ 2015;22:509–16.
36. Wu C. Chromatin remodeling and the control of gene expression. J Biol Chem 1997;272:28171–4.
37. Masumi A, Ozato K. Coactivator p300 acetylates the interferon regulatory factor-2 in U937 cells following phorbol ester treatment. J Biol Chem 2001;276:20973–80.
38. Seiler N. Catabolism of polyamines. Amino Acids 2004;26:217–33.
39. Mudumba S, Menezes A, Fries D, Blankenship J. Differentiation of PC12 cells induced by N8-acetylspermidine and by N8-acetylspermidine deacetylase inhibition. Biochem Pharmacol 2002; 63:2011–18.
40. Dhalluin C, Carlson JE, Zeng L, et al. Structure and ligand of a histone acetyltransferase bromodomain. Nature 1999;399:491–6.
41. Hudson BP, Martinez-Yamout MA, Dyson HJ, Wright PE. Solution structure and acetyl-lysine binding activity of the GCN5 bromodomain. J Mol Biol 2000;304:355–70.