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Gcn5p is involved in the acetylation of histone H3 in nucleosomes

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Abstract Enzymatic extracts from a *gcn5* mutant and wild-type strains of *Saccharomyces cerevisiae* were chromatographically fractionated and the histone acetyltransferase activities compared. When free histones were used as substrate, extracts from wild-type cells showed two peaks of activity on histone H3 but extracts from *gcn5* mutant cells showed only one. With nucleosomes as substrate, the histone acetyltransferase activities present in extracts from the *gcn5* mutant strain were not able to modify H3 whereas wild-type cell extracts acetylated intensely this histone. The activity that acetylated nucleosome-bound H3 behaved as a 170-kDa complex. We suggest that Gcn5p represents a catalytic subunit within a multiprotein complex containing proteins that confer on it the ability to acetylate H3 in nucleosomes.

Key words: Chromatin; Acetylation; Histone acetyltransferase, *GCN5*; Nucleosome; *Saccharomyces cerevisiae*

1. Introduction

The reversible acetylation of lysine residues in nucleosome core histones is a salient process in eukaryotic cells. First, this post-translational modification occurs in all known eukaryotic organisms. Second, and most importantly, it correlates with several essential events during the life cycle of the eukaryotic cell, gene activation being the one that has attracted most attention. The relationship between histone acetylation and gene transcription has been suggested to occur for more than 20 years (reviewed in [1–5]) although the precise mechanisms that link histone acetylation with transcriptional activity remain obscure. Histone acetylation is also involved in processes such as nucleosome assembly during DNA replication, the replacement of histones by protamines during spermatogenesis and DNA repair [1]. How to explain the role of this post-translational modification in so different and vital cellular processes? Some authors have proposed that histone acetylation deorganizes high-order structures in chromatin and thus, DNA results more accessible to different *trans*-acting factors [6]. Nevertheless, there are several lines of evidence indicating that the acetylation of some histones or even of specific lysine residues is related to a particular function [7–14]. In other words, it is possible that different patterns of lysine acetylation could be related to different specific functions. The selection of acetylation sites could be accomplished either by a set of histone acetyltransferase (HAT) enzymes with different specificities, or by a reduced number of enzymes which discriminate between the lysines by means of a fine-tuning performed by additional factors. The yeast protein encoded by *GCN5* gene, previously described as a transcriptional adaptor required by a group of transcriptional activators ([15], reviewed in [16]), has been recently identified as the catalytic subunit of a yeast HAT-A [17]. Expression of recombinant Gcn5p (rGcn5p) produces a HAT activity that modifies H3 and to a lesser extent H4 in their free forms, but surprisingly it does not modify any nucleosome-bound histone [18,19]. The rGcn5p activity has been related to transcriptional regulation because it specifically acetylates lysine 14 from H3 and lysines 8 and 16 from H4 [18], whereas during chromatin assembly the acetylated lysines are in position 9 of H3 [18] and 5 and 12 of H4 [14]. In our laboratory it has been previously reported that the yeast *Saccharomyces cerevisiae* contains at least four histone acetyltransferase activities [20]. Three of these enzymes are type A, located in nuclei and able to acetylate nucleosome-bound histones, albeit each one shows a different preference for the histone substrate [21].

In this paper we compare the HAT activities obtained by biochemical methods from *gcn5* mutant and wild-type yeast strains. Yeast *gcn5* mutant extracts contain a reduced enzymatic activity towards free histone H3 but, more importantly, are completely unable to acetylate H3 in nucleosomes. We also show that the Gcn5p associated HAT activity has a molecular mass of about 170 kDa, notably higher that the one reported for Gcn5p (50.5 kDa) [17].

2. Materials and methods

2.1. Strains, cultures and preparation of cell extracts

Yeast strains PSY316 (*MATα, ade2-101; leu2-3,112, his3-Δ200 lys2-801; and ura3-52*; wild-type) and GMY27, a *gcn5* mutant (*GCN5::HisG*) isogenic to PSY316, employed in this work were kindly supplied by Dr. N. Silverman (M.I.T., Cambridge, MA, USA). Yeast cells were grown at 28°C to exponential phase in liquid YEPG medium and they were spheroplasted by zymolyase digestion [22]. Crude enzymatic extracts were obtained by the salt-dissociation/ultracentrifugation method previously described [20] with slight modifications. Spheroplasts were lysed in buffer A (75 mM Tris-HCl, pH 7.9, 0.25 mM EDTA, 5 mM 2-mercaptoethanol, 0.05% (v/v) Tween 20, 1 mM phenylmethylsulfonyl fluoride and 2 μM *trans*-epoxysuccinyl-L-leucylamido-(4-guanidino)butane) and solid NaCl was added to give a final concentration of 0.5 M. The mixture was stirred for 30 min and it was centrifuged at 100000×*g* for 1 h. The supernatant, containing crude acetyltransferases, was dialysed against buffer B (15 mM Tris-HCl, pH 7.9, 0.25 mM EDTA, 5 mM 2-mercaptoethanol, 0.05% (v/v) Tween 20, 10% (v/v) glycerol, 10 mM NaCl) and used immediately for chromatographic fractionation (see below).

2.2. Preparation of histone and nucleosome substrates

Yeast and chicken erythrocyte histones were obtained as described elsewhere [22]. Chicken erythrocyte nucleosomes were prepared by micrococcal nuclease digestion of nuclei. Briefly, purified nuclei were suspended in digestion buffer (15 mM Tris-HCl, pH 7.9, 10 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10% (v/v) glycerol, 0.25 M sucrose, 1 mM phenylmethylsulfonyl fluoride and 2 μM *trans*-epoxysuccinyl-L-leucylamido-(4-guanidino)butane) to a concentration of about 100 units of A_{260} per ml. Micrococcal nuclease (Boehringer) was then added (300 U/ml) and the suspension was incubated for 25 min at 37°C. Reaction was stopped by adding EDTA to 10 mM and chilling

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on ice. The mixture was then dialysed against 15 mM Tris-HCl, pH 7.5, 0.25 mM EDTA and centrifuged at 17000×*g* for 20 min. The supernatant, containing soluble nucleosomes (about 3 mg of DNA/ml as determined by absorption at 260 nm), was saved and used, without any further purification, as substrate for the acetyltransferases. 1.8% agarose electrophoretic analysis [23] revealed that 80% of DNA was indeed in the form of oligonucleosomes ($n \leq 5$).

2.3. Fractionation of enzymes

Ion-exchange chromatographies were carried out by the procedure previously described [22], using Q-Sepharose FF (Pharmacia) instead of DEAE-Sepharose. In order to compare the content of histone acetyltransferase activity in wild-type and *gcn5* mutant yeast strains, two identical Q-Sepharose FF columns (7×1 cm) were simultaneously used. Crude enzyme extracts, prepared from wild-type and *gcn5* mutant strains and containing similar amount of proteins (determined by absorption at 280 nm), were loaded onto the columns equilibrated with buffer B. Non-retained materials were removed by washing with the same buffer until A_{280} was below 0.05 units. Elution of bound proteins was performed with 60 ml of a linear gradient from 10 to 500 mM NaCl in buffer B at a flow of 100 ml/h. Fractions of 2 ml were collected and assayed for histone acetyltransferase activity.

The molecular masses of HAT enzyme complexes were estimated by ultracentrifugation using a linear sucrose gradient (7–18%) in 15 mM Tris-HCl, pH 7.9, 0.25 mM EDTA, 20 mM NaCl. Q-Sepharose chromatography fractions with Gcn5p-associated HAT activity were concentrated by ultrafiltration in an Amicon cell (molecular mass cut-off 30 kDa) and an aliquot of 0.25 ml was layered on the top of an 11 ml gradient and centrifuged in a Beckman SW 41 rotor at 40000 rpm for 20 h. After centrifuging, the tubes were bottom-punctured, and fractions of 0.7 ml were recovered and assayed for histone acetyltransferase activity.

2.4. Enzymatic assays

Histone acetyltransferase activity was assayed using chicken erythrocyte (and yeast) histones and [1-^{14}C]acetyl CoA (55 mCi/mmol,

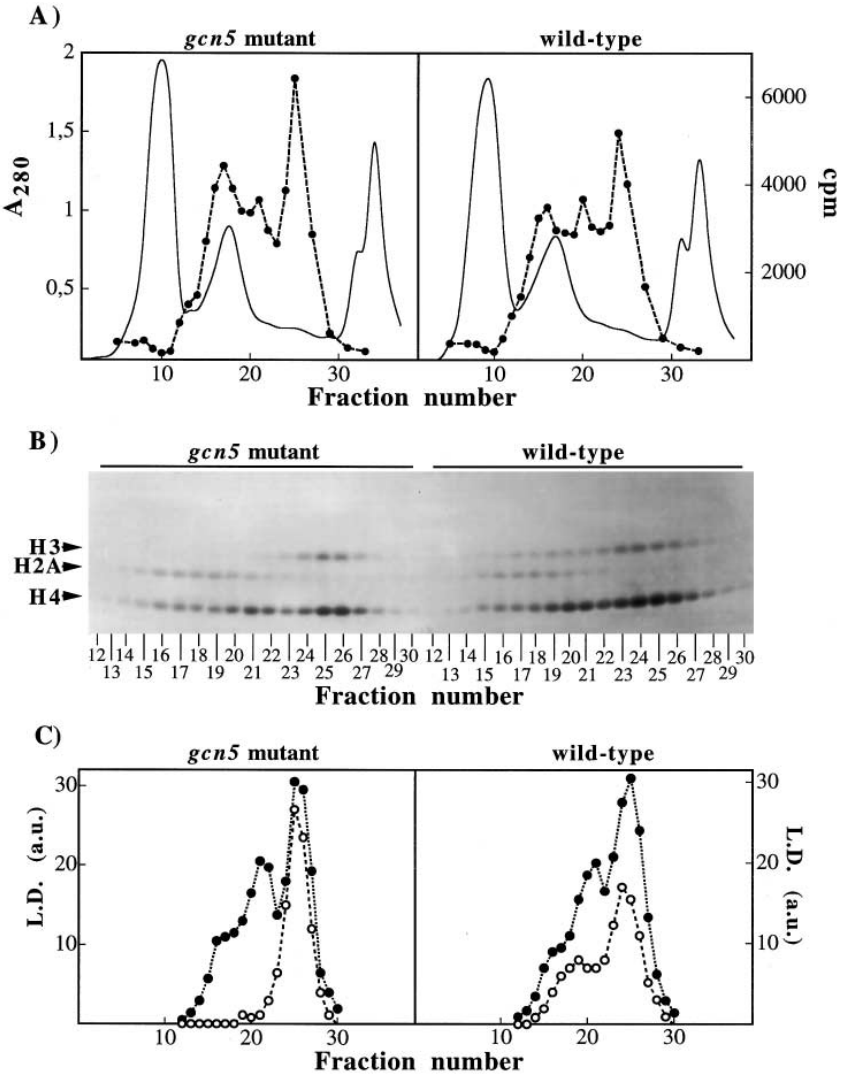

Fig. 1. Analysis of histone acetyltransferase activities in wild-type and *gcn5* mutant yeast strains. A: Chromatographic profiles of yeast histone acetyltransferase activities. Yeast extracts from PSY316 (wild-type, right panel) and GMY27 (*gcn5* mutant, left panel) strains were chromatographied on Q-Sepharose FF columns. Retained proteins were eluted using a linear NaCl gradient (10–500 mM) and the collected fractions were assayed for histone acetyltransferase activity with chicken free whole histones. Enzyme activity is expressed as cpm (●); protein concentration was monitored by A_{280} measurement (solid line). B: Histone specificity analysis of yeast histone acetyltransferases. The assay was carried out by incubating free chicken whole histones with [1-^{14}C]acetyl CoA in the presence of the fractions identified by their numbers. Histones were resolved by SDS-PAGE and the gels were subjected to fluorography. Only the fluorograms are shown. The positions of H3, H2A and H4 are indicated. C: Analysis of the acetylating activities towards individual histones. Radiolabel incorporated into each histone was determined by densitometric analysis of the fluorograms with a 2202 Ultroscan (LKB) densitometer. The labeling density (L.D.) values expressed in arbitrary units (a.u.) were plotted against fraction number. (○) L.D. for H3; (●) 0.5×L.D. for H4.

Amersham) as described [22]. Enzymatic assays with nucleosomes were carried out with chromatographic fractions previously dialysed (against buffer B, 20 mM NaCl) to eliminate the salt used for the elution. In this instance, enough volume of the nucleosome stock preparation was added to the enzyme fractions to obtain a final histone concentration (about 1 mg/ml) similar to that used in free histone assays. After incubation, reaction mixtures were made 0.25 M in HCl using a stock solution of 3 M HCl, chilled on ice and spun at $10000 \times g$ for 10 min in a microcentrifuge. Supernatants containing histones were recovered and subsequently analyzed as described for free histones.

Determination of histone specificity of acetyltransferases was carried out with 15% SDS-PAGE, Coomassie blue staining and fluorography, exactly as described [22].

3. Results and discussion

3.1. The gcn5 mutant retains most HAT activity

We obtained crude enzymatic extracts from yeast *gcn5* mutant and wild-type strains by a previously described method [20] which proved to be very efficient in recovering whole HAT activity. Fig. 1A shows the HAT activity profiles obtained from both mutant and wild-type yeast strain extracts after fractionation on Q-Sepharose and using free histones as substrate. The enzymatic assays were carried out with chicken instead of yeast free histones for a better comparison with the results obtained when nucleosome-bound histones were used as substrate (see below). In any case, similar results were obtained with yeast free histones (data not shown). As we used the same amount of protein extract in both chromatographies, the comparison of the activity profiles indicates that there are no significant changes in total HAT activity in the *gcn5* mutant. There are only slight differences in the relative proportion of the three peaks obtained in both cases. This result indicates that Gcn5p actually represents a small fraction of the HAT activity present in yeast cells. This supposition was confirmed by analysis of the incorporation of acetyl groups into free histones as shown in the fluorogram of Fig. 1B. The only difference between the two profiles is the acetylation of histone H3 in fractions 16–21 which is not present in *gcn5* mutant strain. The label observed on the other histones is very similar in both chromatographies. Fig. 1C shows the densitometric profiles of radioactivity label on histones H3 and H4. Label on histone H2A is not relevant to this research

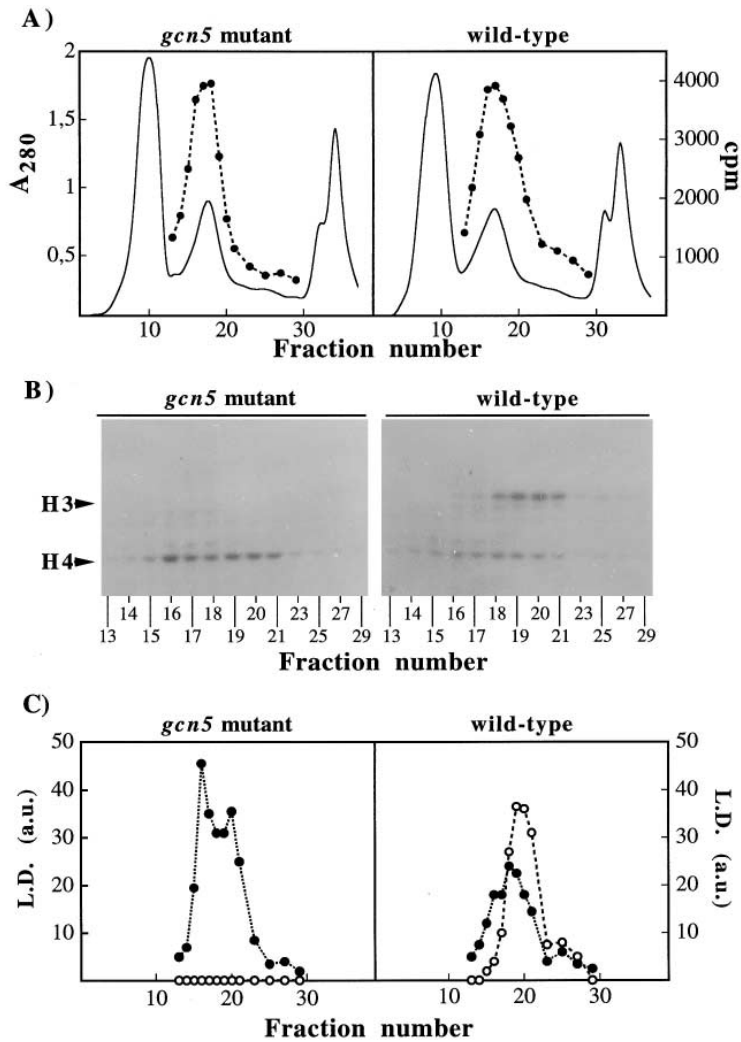

Fig. 2. Nucleosome acetylating activities in wild-type and *gcn5* mutant yeast strains. A: Profiles of nucleosome acetylating activity from Q-Sepharose chromatography. Fractions from the Q-Sepharose chromatography were dialysed and assayed using chicken erythrocyte nucleosomes; wild-type (right panel) and *gcn5* mutant (left panel). Symbols are as in Fig. 1. B: Histone specificity of yeast nucleosome acetyltransferases. Assay conditions, electrophoresis and fluorography were as described for free histones. C: Densitometric analysis of histone specificity when nucleosomes were used as substrate. (○) L.D. for H3; (●) L.D. for H4.

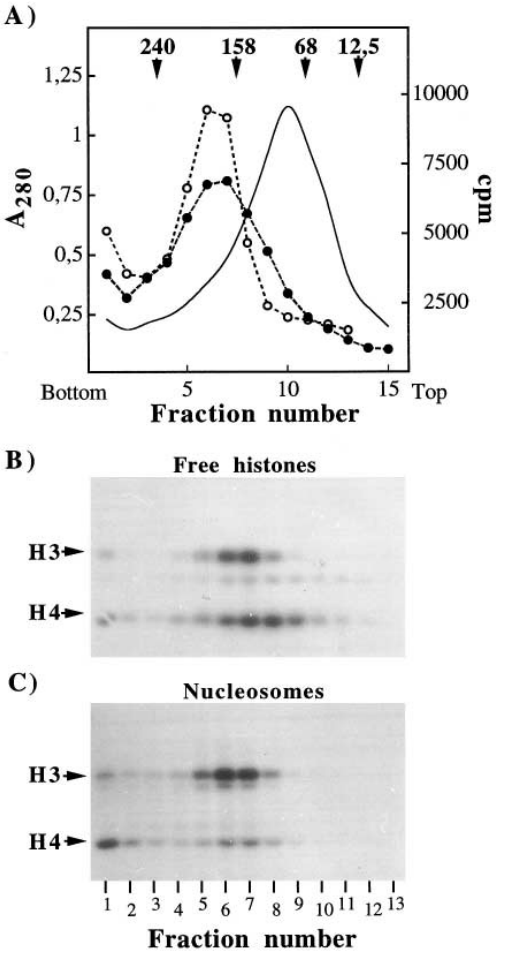

Fig. 3. Determination of the molecular mass of HAT enzyme containing Gcn5p by sucrose gradient ultracentrifugation. A: Q-Sepharose fractions with Gcn5p-associated HAT activity were concentrated, loaded on a linear 7–18% sucrose gradient and centrifuged at 40 000 rpm for 20 h. Fractions of 0.7 ml were recovered and their absorbance at 280 nm (solid line) and histone acetyltransferase activity, using chicken free histones (●) and nucleosomes (○) were determined. Arrows show the positions of the markers used for gradient calibration: catalase, 240 kDa; aldolase, 158 kDa; BSA, 68 kDa; and cytochrome *c*, 12.5 kDa. The position of enzyme containing Gcn5p into the gradient was determined by its efficiency of H3 acetylation (monitored by fluorography) when free histones (B) and nucleosomes (C) were used as substrates. The migration of H3 and H4 histones is indicated in the fluorograms.

and it is not shown in order to clarify the figure. The comparison of the densitograms shows that *gcn5* mutant has lost one of the two HAT activity peaks on free H3 presents in the elution gradient. This result agrees with the clear substrate preference of rGcn5p for histone H3 [18], but on the other hand demonstrates that this is not the only yeast histone acetyltransferase able to modify this histone.

Two HAT activities that acetylate both chicken erythrocyte and yeast free histone H3, designated HAT-A2 and HAT-A3 after their elution order in anion exchange chromatography, have been described in our laboratory [21]. Because of its behavior on Q-Sepharose chromatography and its histone specificity, we propose that Gcn5p is the catalytic subunit of yeast HAT-A2 enzyme. The analysis presented in this work corroborates the existence of different histone acetyltransferases with different specificities and, probably, different biological functions in yeast.

3.2. *Gcn5p is required for nucleosome-bound H3 acetylation*

Although it has been suggested that Gcn5p is related to transcriptional regulation [17], the expression of recombinant Gcn5p (rGcn5p) produces a HAT activity unable to acetylate any nucleosome-bound histone [18,19]. On the other hand, HAT-A2 specifically acetylates H3 in nucleosomes [21]. In order to clarify this apparent contradiction, we performed a similar experiment to that presented in Fig. 1, but using nucleosomes as substrate. In this instance, enzymatic assays were carried out with chicken instead of yeast nucleosomes due to the instability of the latter [24] and the difficulty to obtain them in a pure form. As shown in Fig. 2A, HAT activity profiles from both extracts using nucleosome-bound histones are also very similar, albeit the total amount of radioactivity incorporated is 15% higher in wild-type cell extracts when compared to mutant cell extracts. However, when the radioactivity incorporation into individual histones was analyzed, it was obvious that H3 was not acetylated in *gcn5* mutant cell extracts, whereas it was intensely acetylated in extracts from wild-type cells (Fig. 2B,C). Thus Gcn5p is indispensable for the acetylatation of H3 in nucleosomes and, hence, Gcn5p should participate in the HAT activity that acetylates nucleosome-bound H3 in yeast cells. This result is also consistent with our proposal that Gcn5p takes part of HAT-A2.

3.3. *Gcn5p acts on nucleosomes as a high molecular mass complex*

In order to determine the molecular size of the HAT activity towards nucleosome-bound H3, fractions containing Gcn5p-associated HAT activity were pooled (fractions 16–21 in Fig. 2) and analyzed by ultracentrifugation in sucrose gradients. Fig. 3A shows that the maximum HAT activity is found in fractions corresponding to a molecular mass of around 170 kDa. We also performed the assay and fluorography with the different gradient fractions using free (Fig. 3B) or nucleosome-bound (Fig. 3C) histones as substrate. In the assay with free histones a small contamination with the HAT activity working on H2A is observed, which shows a molecular mass slightly smaller than the activity that acetylates H3. The presence of labeled H2A in both wild-type and mutant cell extracts indicated that Gcn5p does not participate in this activity (see Fig. 1B). On the other hand, the densitometric analysis of labeled H3 and H4 does not make it possible to discern whether the complex formed by Gcn5p and other proteins acetylates H4 as well as H3. This complex heavily acetylates nucleosome-bound H3, as shown in Fig. 3C, although the possibility exists that it also acts on H4 and H2B in nucleosomes as indicated by the slight label on these histones in the same fractions. However, when the sucrose gradient fractionation is performed with the equivalent Q-Sepharose fractions obtained from the mutant cell extracts, H4 and H2B also appeared labeled (data not shown). The molecular mass of the complex (170 kDa) is notably higher than the molecular mass proposed for Gcn5p (50.5 kDa) [17]. Most likely, Gcn5p is a catalytic subunit belonging to a multiprotein complex with HAT activity. This complex should contain several subunits that confer on Gcn5p the ability to acetylate histones in nucleosomes being thus able to modify chromatin. This conclusion agrees with the well established observation

that Gcn5p functions as a complex with two other proteins, the coactivators Ada2p and Ada3p [25–27]. Recently, Roth and Allis [28] have proposed that Gcn5p could participate in complexes with one or several of the following proteins: Ada1p, Ada2p, Ada3p, and Ada5p. The whole complex, with a predicted molecular mass of 295 kDa in yeast, could associate with nucleosomal structures, whereas the other complexes with only some of the proteins could recognize H2A-H2B dimers or $(H3\text{-}H4)_2$ tetramers. However, our results show that a complex of only 170 kDa acetylates nucleosomes in vitro. We are presently studying the presence of these proteins in the enzymatic complex with HAT activity to determine the factors conferring on Gcn5p the ability to acetylate nucleosomes.

In summary, we have demonstrated that yeast Gcn5p participates in a complex with HAT activity that modifies H3 in nucleosomes.

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References

[1] Csordas, A. (1990) Biochem. J. 265, 23–38.
[2] Tordera, V., Sendra, R. and Pérez-Ortín, J.E. (1993) Experientia 49, 780–788.
[3] Loidl, P. (1994) Chromosoma 103, 441–449.
[4] Turner, B.M and O'Neill, L.P. (1995) Semin. Cell Biol. 6, 229–236.
[5] Brownell, J.E. and Allis, C.D. (1996) Curr. Opin. Genet. Dev. 6, 176–184.
[6] Wolffe, A.P. and Pruss, D. (1996) Cell 84, 817–819.
[7] Pesis, K.H. and Matthews, H.R. (1986) Arch. Biochem. Biophys. 251, 665–673.
[8] Loidl, P. and Gröbner, P. (1987) Nucleic Acids Res. 15, 8351–8366.
[9] Kayne, P.S., Kim, U.J., Han, M., Mullen, J.R., Yoshizaki, F. and Grunstein, M. (1988) Cell 55, 27–39.
[10] Durrin, L.K., Mann, R.K., Kayne, P.S. and Grunstein, M. (1991) Cell 65, 1023–1031.
[11] Turner, B.M., Birley, A.J. and Lavender, J. (1992) Cell 69, 375–384.
[12] Thompson, J.S., Ling, X. and Grunstein, M. (1994) Nature 369, 245–247.
[13] Sobel, R.E., Cook, R.G. and Allis, C.D. (1994) J. Biol. Chem. 269, 18576–18582.
[14] Sobel, R.E., Cook, R.G., Perry, C.A., Annunziato, A.T. and Allis, C.D. (1995) Proc. Natl. Acad. Sci. USA 92, 1237–1241.
[15] Georgakopoulos, T. and Thireos, G. (1992) EMBO J. 11, 4145–4152.
[16] Guarente, L. (1995) Trends Biochem. Sci. 20, 516–521.
[17] Brownell, J.E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D.G., Roth, S.Y. and Allis, C.D. (1996) Cell 84, 843–851.
[18] Kuo, M.-H., Brownell, J.E., Sobel, R.E., Ranalli, T.A., Cook, R.G., Edmondson, D.G., Roth, S.Y. and Allis, C.D. (1996) Nature 383, 269–271.
[19] Yang, X.-J., Ogryzko, V.V., Nishikawa, J.-I., Howard, B.H. and Nakatani, Y. (1996) Nature 382, 319–324.
[20] López-Rodas, G., Tordera, V., Sánchez del Pino, M.M. and Franco, L. (1989) J. Biol. Chem. 264, 19028–19033.
[21] López-Rodas, G., Tordera, V., Sánchez del Pino, M.M. and Franco, L. (1991) Biochemistry 30, 3728–3732.
[22] López-Rodas, G., Pérez-Ortín, J.E., Tordera, V., Salvador, M.L. and Franco, L. (1985) Arch. Biochem. Biophys. 239, 184–190.
[23] Estruch, F., Pérez-Ortín, J.E. and Franco, L. (1986) Cell. Mol. Biol. 32, 195–199.
[24] Lee, K.P., Baxter, H.J., Guillemette, J.G., Lawford, H.G. and Lewis, P.N. (1982) Can. J. Biochem. 60, 379–388.
[25] Marcus, G., Silverman, N., Berger, S., Horiuchi, J. and Guarente, L. (1994) EMBO J. 13, 4807–4815.
[26] Horiuchi, J., Silverman, N., Marcus, G. and Guarente, L. (1995) Mol. Cell Biol. 15, 1203–1209.
[27] Candau, R. and Berger, S.L. (1996) J. Biol. Chem. 271, 5237–5245.
[28] Roth, S.Y. and Allis, C.D. (1996) Trends Cell Biol. 6, 371–375.