MODIFICATION OF HISTONES DURING THE MITOTIC AND MEIOTIC CYCLE OF YEAST

Brigitte MARIAN and Ulrike WINTERSBERGER
Institute for Cancer Research, University of Vienna, 1090 Vienna, Austria

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1. Introduction

Core histones (H2a,H2b,H3,H4) of yeast have been shown to be similar to histones from higher organisms [1] whereas a protein corresponding to histone H1 seems to be absent from this simple unicellular eukaryote [2]. Also the organization of yeast chromatin into nucleosomes closely resembles that of higher eukaryotes [1]. Histones of other organisms have been found to be subject to various modifications. Phosphorylation, especially that of histones H1 and H3, occurs predominantly during chromosome condensation for mitosis in higher organisms [3] as well as in the slime mold, *Physarum polycephalum* [4]. Phosphorylation of histone H2a was suggested to be involved in interphase heterochromatin structure in a cell cycle-independent manner [5–7]. Highly acetylated histone H4 was discovered specifically in transcriptionally active, DNase I-sensitive, chromatin [8–10], its concentration is inversely correlated with the initiation of chromosome condensation in *Physarum* [11].

We asked whether such modifications, probably being devices for the modulation of DNA–histone interactions, also occurred in yeast and whether appearance of modified histone proteins varied during the vegetative cell cycle or during the sporulation process. We found incorporation of radioactive phosphate into yeast histone H2a and to a much smaller amount into H2b, but not into H3 and H4. The degree of incorporation did not change substantially during the vegetative growth cycle but increased considerably in cells preparing for sporulation. Radioactive sodium acetate was found to be incorporated into all core histone-bands. Analysis of unlabeled histones in acid–urea gels, in which the acetylated variants of histones H4 and H3 can be separated, showed that these 2 histones in yeast exist as several subspecies acetylated to different degrees. Variations in the relative amounts of these subspecies were found during the mitotic cell cycle and less pronounced during the process of sporulation induction.

2. Experimental

Cells of the diploid strain A 1160 of *Saccharomyces cerevisiae* were synchronized as in [12]. They were inoculated at ~10^7 cells/ml into complete medium (0.5% peptone, 0.3% yeast extract, 1% glucose) and were grown at 30°C. Synchronous growth was monitored by determining the bud index and samples (100 ml each) for pulse labeling with [^{32}P]phosphoric acid (25 μCi/ml) or with tritiated sodium acetate (10 μCi/ml) were taken at different time points after the first cell cycle was finished. After 15 min of radioactive labeling the samples were processed as in [12]. When measuring acetate incorporation, 100 mM sodium butyrate was added for suppression of histone deacetylation [13]. Sporulating cells were obtained as in [2]; they were pulse-labeled with [^{32}P]phosphoric acid in the same way as vegetatively growing cells. Histone proteins from samples of both mitotic and meiotic cells were isolated from cell nuclei purified via spheroplasts according to the procedure given in [12]. When unlabeled histone proteins were analyzed for acetylated subspecies, samples of 500 ml were taken and spheroplasts were prepared in the absence of butyrate, buffers used during steps after lysis of spheroplasts, however, contained 100 mM sodium-butyrate to avoid histone-deacetylation during preparation. Care was taken that all samples were processed identically (especially concerning the time of converting cells into spheroplasts) and as rapidly as possible. Electrophoretic analyses of histone proteins were done in SDS–polyacrylamide gels as in [2] and

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in acid–urea gels containing 6.25 M urea according to [14]. The gels were stained for protein with Coomassie blue, individual ^{32}P-labeled bands were visualized by autoradiography, [^{3}H]acetate containing bands after fluorography of gels as in [2]. Gels and films were scanned and peak areas were calculated.

3. Results

As shown in fig.1 incorporation of [^{3}H]acetate as well as [^{32}P]phosphate into histone proteins separated by SDS–polyacrylamide gel electrophoresis could be detected in logarithmically growing yeast cells. Radioactive acetate labeled all 3 core histone bands (lane b), radioactivity resulting from phosphorylation was found only in the histone H2a band and to a much smaller amount in the band representing H3 and H2b but not in H4 (c). By analysis of phosphate-labeled histones in an acid–urea gel (fig.1d,e) in which H3 is separated from H2a and H2b it could be decided that the small amount of label detected in the H3/H2b band of the SDS gel could only come from histone H2b because the area of the H3 band was found free of any radioactivity (fig.1e). To learn more about the possible role of histone modifications in yeast we followed the patterns of phosphorylation and acetylation during the mitotic cycle of synchronized cells as well as during the induction of sporulation in nitrogen-free medium.

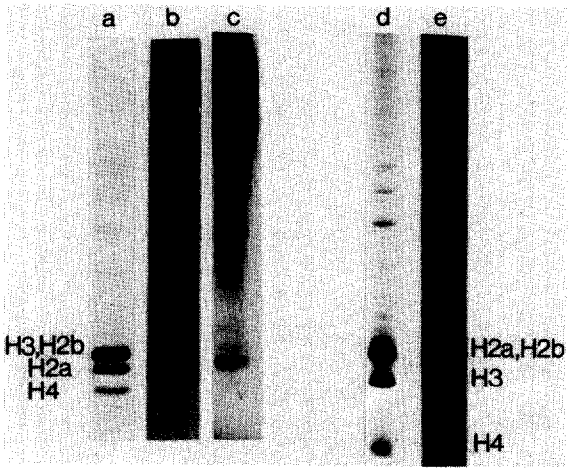

Fig.1. Incorporation of sodium [^{3}H]acetate and [^{32}P]phosphoric acid into histones from logarithmically growing yeast cells. Histone proteins were labeled and isolated from purified chromatin. Lanes (a–c): SDS–polyacrylamide gel electrophoresis, protein bands stained with Coomassie blue (a); incorporation of [^{3}H]acetate visualized by fluorography (b); and [^{32}P]phosphate labeling by autoradiography (c). Lanes (d,e): Acid–urea gel electrophoresis of histones purified in the absence of sodium butyrate, protein stain (d) and autoradiography of ^{32}P-labeled histones (e).

3.1. *Mitotic cell cycle*

Yeast cells, synchronously growing in full medium, were pulse-labeled with [^{32}P]phosphate or [^{3}H]acetate during the G1-, S- and G2/mitosis-phases of the cell-cycle. We found no significant difference in the rate of the phosphate-labeling of the histone fraction between the 3 phases (2 expt) (table 1). Acetate-labeling, representing the turnover of histone-acetylation during the pulse times, consistently showed small reproducible variations (2 expt, fig.2A). The labeling rate decreased by ~25% after cells had passed the S-phase and rose again during the next G1 period. We also analyzed unlabeled histones from cells at the 3 stages in acid–urea gels. This allowed a calculation of the relative amounts of the individual acetylated subspecies of histone H4 from scans of the gels. Variations

Table 1
Relative specific activity of ^{32}P-labeled histone proteins at different stages of the vegetative cell cycle and of the sporulation process

	Stage	Relative spec. act.[a]
Vegetative cycle	G1	1.0
	S	1.2
	G2/M	1.1
Time in sporulation medium (h)	0	1.0
	2.5	3.2
	6	9.1
	9	9.5

[a] ^{32}P-Labeled histone-fractions were separated in SDS–polyacrylamide gels which were stained for proteins with Coomassie blue and scanned. Labeled bands, detected by autoradiography, were also scanned. Protein- and radioactivity peak areas of histone proteins were calculated, from which the specific activity was determined. In addition 2 parallel cultures were grown (during the mitotic cycle as well as throughout sporulation), one labeled continuously, the other used for 10 min pulses with [^{32}P]phosphate. The relationship between the trichloroacetic acid-soluble radioactivity of an aliquot of washed cells from the pulse labeled to that of cells from the continuously labeled culture was defined as specific activity of the soluble phosphate pool at the particular time point. Phosphate incorporation into histones was corrected for changes in this activity

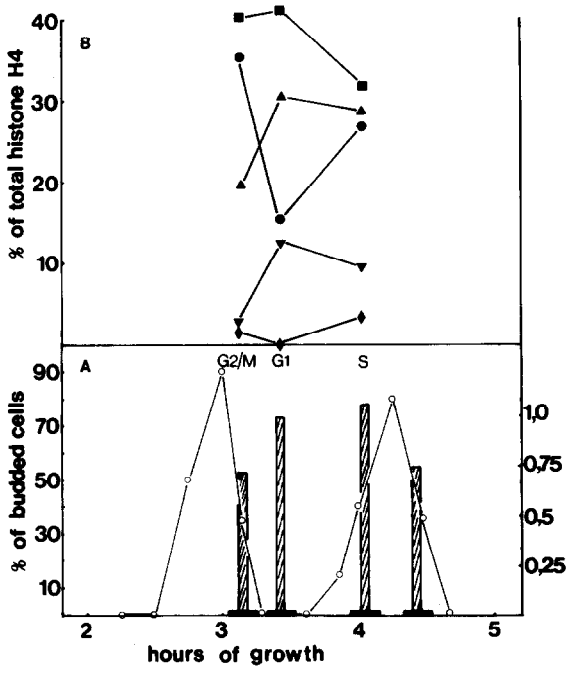

Fig.2. Acetylation of histones during the cell cycle. (A) Incorporation of [^{3}H]acetate into the histone fractions: (o——o) % of budded cells; (▨) relative amount of acetate-label in the histone fractions. Pulse-labeled histones were isolated from nuclei as in section 2, cpm/mg protein were determined and the number obtained for G1-phase histones was arbitrarily set as 1; (▬) pulse times. (B) Acetylated H4 through the cell cycle. The amounts of the individual H4 bands were calculated from scans of acid–urea gels and are depicted as percentage of total H4; percentage of H4 in the non-acetylated (●——●), mono-acetylated (■——■), di-acetylated (♦——♦), tri-acetylated (▼——▼) and tetra-acetylated (▲——▲) form.

occur during the cell cycle (fig.2B). If, in analogy to mammalian cells, we assume that the H4 peaks represent the non-acetylated, the mono-, di-, tri- and tetra-acetylated forms of the histone H4 protein, we can describe our findings as follows: Non-acetylated H4 was found high in cells which are in G2 or mitosis and low during the G1 phase. The mono-acetylated form was predominant during all stages, whereas the di-acetylated form was not detectable in G1 and was present only in low concentration during the rest of the cell cycle. The concentrations of the tri- and tetra-acetylated forms both were highest during the G1-phase and lower during G2/mitosis, tetra-acetylated H4 being a relatively abundant species throughout the whole cell cycle.

3.2. *Sporulation*

Cells were grown in acetate-containing pre-sporulation medium up to 5 X 10^7 cells/ml and then transferred to nitrogen-free sporulation medium. At different times after this transfer, cells were pulse-labeled for 15 min with [^{32}P]phosphate and relative amounts of radioactivity in the histone fractions were determined after electrophoretic separation. To monitor the progress of the early phase of sporulation, premeiotic DNA-synthesis was measured. Table 1 shows that the rate of phosphate incorporation into the histone fraction (mainly into histone H2a) increased considerably, especially at 6 h after transfer when premeiotic DNA synthesis was finished in most of the cells. The presence of acetylated histone species, especially those of H4, throughout the early phase of sporulation was studied by the aid of acid–urea gels (fig.3). As in the vegetatively growing cells, 4 subspecies of histone H4 acetylated to different degrees were detected in addition to the non-acetylated form. The relative amounts of these subspecies at different stages of the sporulation process were calculated and are depicted in fig.4. Whereas the portions of the mono-, di- and tri-acetylated forms did not vary much, the amount of non-acetylated H4 was particularly low 2 h after transfer of cells to nitrogen-free medium

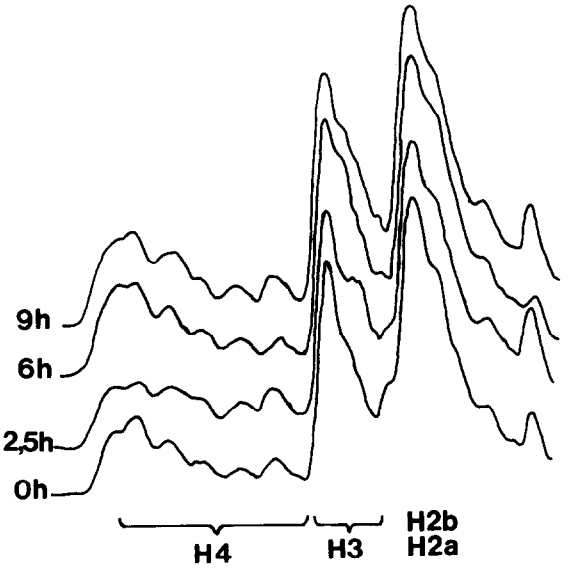

Fig.3. Acid–urea gel electrophoresis of histones from yeast cells harvested at different time points after transfer to sporulation medium. Gels were stained with Coomassie blue and traces were obtained with a Helena Quick Scan-scanner.

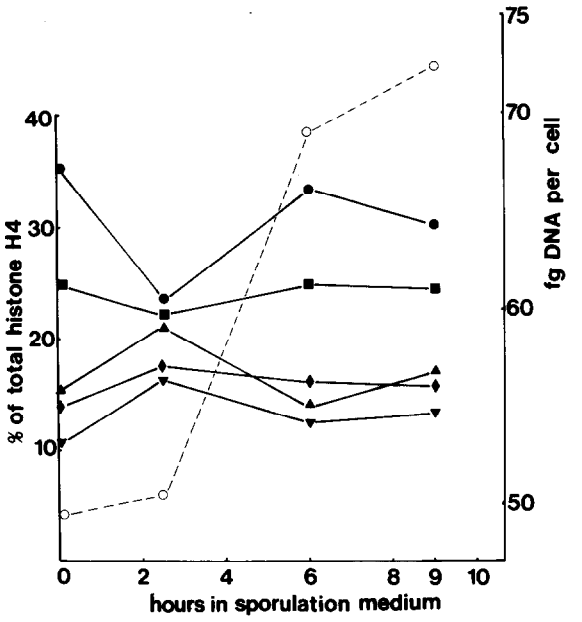

Fig.4. Acetylated H4 species throughout the sporulation process of yeast: (o---o) premeiotic DNA synthesis; percentage of total H4 in differently acetylated forms; for symbols see legend to fig.2.

when premeiotic DNA synthesis started. At this time-point we observed the highest portion of tetra-acetylated histone H4.

4. Discussion

The results of our study show that modifications of histone proteins by phosphorylation and acetylation occur in the simple eukaryote, *Saccharomyces cerevisiae*. The core histone H2a was found to be the main target of phosphorylation. There was no phosphate incorporation into histone H3 which, together with H1, is phosphorylated during chromosome condensation in higher organisms. The fact that a protein which could be unambiguously identified as histone H1 has not yet been detected in yeast [2] as well as the obvious lack of a phosphorylation mechanism for histone H3 can be taken as an indication for a difference between yeast and higher eukaryotes in the strategy of distribution of chromosomes during cell division. In several studies of vertebrate histones, a high degree of H2a phosphorylation could be correlated with increasing amounts of heterochromatin in interphase nuclei [5–7]. We found that during the sporulation process of yeast, when active chromatin is converted into an inactive form, a pronounced rise in the H2a phosphorylation rate was observed (table 1). Thus our finding adds another example of correlation between the organization of chromatin into a condensed storage form and increased H2a phosphorylation.

While our manuscript was in preparation a generally high degree of acetylation of histones from vegetatively growing yeast cells was reported [15] and a connection of this fact with the high transcriptional activity of yeast chromatin was pointed out. Our results are in accord with [15] but we additionally describe a fluctuation of the pattern of acetylated histone species during synchronous growth of yeast cells. Histone acetylation rate was higher for cells from the G1- and S-phase than for a mixture of G2 and mitotic cells. Alternatively, variations in the deacetylation activity between the different stages of the cell cycle could likewise contribute to the observed fluctuations. The analysis of individual acetylated histone H4 proteins in acid–urea gels (fig.2B) shows that during the G1-phase only 15% of this histone was represented by the acetate-free form for cells growing in rich glucose medium. During S and G2/mitosis non-acetylated H4 was at 25% and 36%, respectively. Among the acetylated forms the mono- and tetra-acetylated H4 were the most abundant, especially in the G1 phase. This pattern is consistent with the concept of a relationship between the high transcriptional activity in the G1 phase and acetylation of histone H4.

Our study of histone acetylation during the early phase of yeast sporulation reveals that, in comparison to results from cells grown in glucose containing complex medium, acetylated histone H4 species were less abundant in cells grown on the meager acetate containing presporulation medium (zero-point of fig.4). Shortly after sporulation induction, when premeiotic DNA synthesis started, we had noticed a peak in the rate of histone synthesis [2]. A high degree of transcription activity was observed by others [16] and we now found that the amount of the non-acetylated histone H4 decreased markedly while that of tri- and tetra-acetylated H4 rose. Later, in parallel to a decline of transcriptional activity, the non-acetylated and the mono-acetylated types predominated again (6 h and 9 h points of fig.4).

In conclusion our results suggest that histone acetylation may play a very similar role in *Saccharomyces cerevisiae* as in other organisms whereas only part of

the known spectrum of histone phosphorylation mechanisms, namely the modification of H2a, seems to occur in this unicellular eukaryote.

Acknowledgements

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