HISTONE SYNTHESIS DURING SPORULATION OF YEAST

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1. Introduction

Cells of the yeast, *Saccharomyces cerevisiae*, can exist in three different states, the two haploid states, mating type 'a' or α, and the diploid state a/α . Whereas a and α cells are capable of mating with each other, a/α cells are able to sporulate and do not mate. These different abilities of cells are genetically governed by the mating type locus which is a fascinating example of a regulatory system functioning by DNA rearrangements as described by the cassette model [1]. Diploid a/α cells can be looked upon as the 'cell lineage' which in contrast to α and a cells (and also to laboratory-constructed α/α or a/a cells) possess the ability to go, after receiving a signal 'starvation', through a meiotic division and to form haploid spores. We asked ourselves whether this process, which has some similarity to terminal cytodifferentiation of committed cells of a certain lineage in a higher organism, is reflected in a change of the histone pattern. Particularly, it was our aim to look for histone H1, the presence or absence of which in vegetatively growing cells could not be unambiguously ascertained [2,3]. Although we did not obtain a more conclusive result concerning H1, we found to our surprise that histone synthesis is uncoupled from premeiotic DNA replication and takes place in the early part of the sporulating process. This is in contrast to the situation in vegetatively growing yeast, in which histone synthesis [3] as well as the appearance of histone messenger RNAs (G. Viehauser, E. Wintersberger, unpublished) are tightly coupled to DNA replication during the S phase of the mitotic cell cycle.

2. Experimental

Yeast cells are grown under vigorous aeration in presporulation medium PSP2 [4] at 30°C, harvested by centrifugation at room temperature, washed with water, resuspended in sporulation medium free of any nitrogen source [4] to 1.5×10^7 cells/ml and incubated at 30°C. From this cell suspension samples of 400 ml were taken at various times and proteins were pulse-labeled with [^{3}H]lysine (1 μCi/ml culture, 40 Ci/mmol) by further incubation for 10 min at 30°C. After labeling, protein synthesis was stopped by addition of cycloheximide (0.2 mg/ml) and nuclei were isolated via spheroplasts essentially as in [2,3]. The nuclear pellet was suspended in ~2 vol. 25 mM NaCl solution containing 10 mM ethylendiaminotetraacetic acid and lysed by addition of an equal amount of this solution containing 1% Nonidet P40. Part of the resulting suspension was used to determine incorporation of lysine-label into total nuclear protein by acid precipitation. Chromatin was purified by centrifugation for 30 min at 20 000 × *g* through a Metrizamide (Nyegaard, Norway) gradient (10–30% in the above NaCl solution). The histones were extracted from the chromatin pellet with 0.2 M sulfuric acid, precipitated with a 10-fold vol. acetone and dried under vacuum. They were solubilized in 62.5 mM Tris–HCl buffer (pH 6.8) and aliquots were used to determine protein concentration according to [5] and ^{3}H-incorporation by spotting 10 μl on Whatman GFC-filter disks which were dried and counted for radioactivity in a toluene scintillator. Incorporation of lysine label into total protein was determined by counting total trichloroacetic acid-precipitable material from aliquots taken from each sheroplast suspension. The amount of [^{3}H]lysine not taken up by the cells during the labeling period was determined by measuring the radioactivity in the supernatant medium after centrifugation of the labeled cells (5 μl supernatant were spotted on a Whatman GFC filter, dried and counted in toluene

scintillator). Separate samples of 100 ml suspension each were used for the determination of the amount of DNA/cell according to the Burton procedure [6]. Asciformation was observed by light microscopy: 1% asci were counted after 10 h; 20% after 21 h; 40% after 26 h; and 70% after 42 h. All buffers contained the protease inhibitors phenylmethylsulfonylfluoride (6 mg/l), Trasylol (6.5 mg/l, gift from Bayer-Pharma, Austria) and Pepstatin (6.5 ml/l, Protein Res. Found., Japan). Electrophoretic analysis was done in 18% SDS–polyacrylamide slab gels by the method in [7]. Labeled histones were treated for fluorography as in [8]. A Kodak RP Royal X-Omat X-ray film was exposed to the dried gel for 10 days at −80°C and subsequently scanned on a Chromoscan (Joyce-Loeble).

3. Results and discussion

Diploid yeast cells were grown in buffered acetate medium (presporulation medium according to [4]) until they had finished their last mitotic division cycle. They were then transferred to a sporulation medium free of any nitrogen source [4]. During the sporulation process we followed the DNA content/cell, the incorporation of radioactively labeled lysine into total cellular, total nuclear and histone protein as well as asci formation. In table 1 all additional parameters which were checked routinely during each experiment are summarized for one experiment. By determining the number of protoplasts, the DNA and protein content of the nuclear pellets as well as the amount of histone protein recovered from 6×10^9 cells for each time point we convinced ourselves that the experimental conditions under which the [^{3}H]lysine incorporation data were obtained were reasonably constant. In fig.1 results of a typical experiment are depicted: diploid a/α cells (strain 419, kindly supplied by G. Simchen) were grown to 8×10^7 cells/ml and harvested when they were unable to go through a further mitosis in the presporulation medium. DNA content/cell started to rise 3 h after transfer to sporulation medium and reached its maximal value after 10 h. Looking at the rate of histone synthesis one notices in fig.1A a definite rise as early as 2 h after the transfer, a peak after 3 h and a decrease from thereon so that histone synthesis after 4 h is back at its low value observed at the time of the transfer to sporulation medium. DNA replication, in contrast, is still fully active at this time In order to correct for possible changes of pool size during the period of the experiment we have also blotted the data as fraction of radioactivity in histones relative to that in total nuclear or spheroplast protein. Curves such as those shown in fig.1A were obtained repeatedly also with a different strain, namely strain AP 3 a/α (kindly supplied by Anita Hopper) so that we conclude that during yeast sporulation histone synthesis is regulated independently from synthesis of total proteins (as well as of total nuclear proteins) and is uncoupled from premeiotic DNA synthesis. Interestingly the time-point of maximal histone synthesis is identical with that of 'readi-

Table 1
Purification of nuclei and histones at different times during sporulation; for each time point 6×10^9 cells were used, conversion into protoplasts was achieved at a yield of ~80–90%

Time after transfer to sporulation medium (h)	Nuclei			Histones	
	μg nuclear DNA recovered/ 6×10^9 cells	%[a]	Relation DNA:protein	μg protein recovered/ 6×10^9 cells	cpm/μg protein
0	230	(70)	1:13	200	103
0.5	250	(76)	1:18	230	76
1	210	(65)	1:15	250	138
2	180	(54)	1:20	200	340
3	200	(51)	1:19	300	425
4	210	(51)	1:17	320	29
5	200	(45)	1:21	360	14
6	280	(52)	1:19	450	9

[a] % Recovery: μg DNA in the nuclear pellet × 100 in relation to the total amount of DNA in 6×10^9 cells as determined by the Burton test

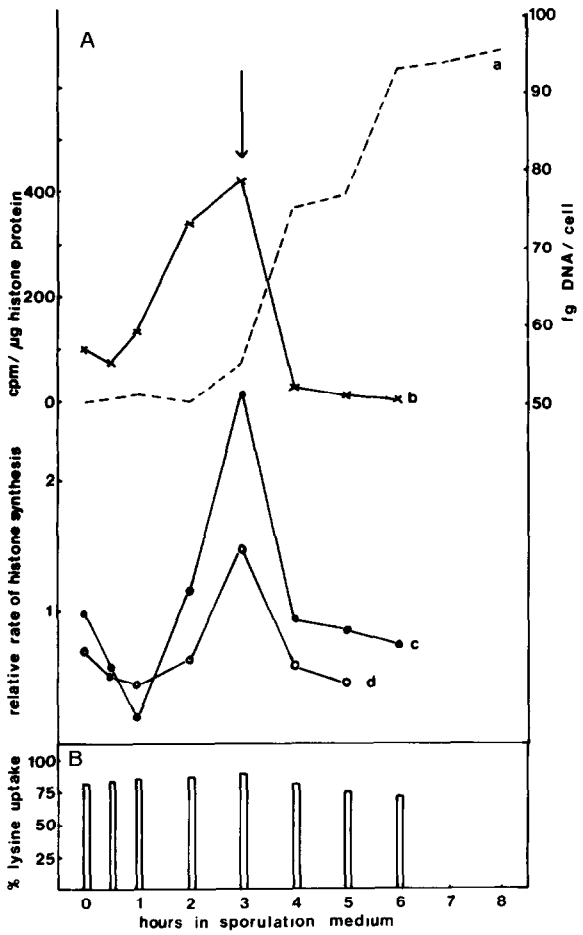

Fig.1. (A) Synthesis of histones during sporulation of *Saccharomyces cerevisiae*: (a) amount of DNA/cell; (b) [^{3}H]-lysine label incorporated into histones; (c) [^{3}H]lysine label in histones relative to that in total nuclear proteins; (d) that in protein of total spheroplasts; (→) time point when cells are ready to sporulation according to [4]. (B) % Labeled lysine taken up by yeast cells during sporulation.

ness to sporulate' as defined [4]. A control experiment was done with strain AP 3 α/α (also obtained from A. Hopper). For this strain histone synthesis rate was found gradually decreasing and no peak was observed during incubation of cells in sporulation medium (not shown). Therefore, in a diploid strain homozygous for the mating type not only premeiotic DNA replication [9] but also the accompanying histone synthesis is absent. To ascertain that our determinations of lysine incorporation did not merely reflect differences in the uptake of the radioactive amino acid by the cells we determined the % of [^{3}H]lysine left in the medium after the incorporation assay. From fig.1B which represents the amount of lysine taken up from the medium by the cells at each time-point, it is obvious that the variations of uptake are small. A measurement of the soluble amino acid pool during yeast sporulation had been done in [9]. The protein precursor pool remained constant throughout the sporulation process [9]. We confirmed that the amount of lysine taken up by the cell did not significantly change the internal pool because the same data were obtained whether 25 or 250 nmol lysine were added to the labeling medium as long as the total radioactivity was kept constant.

We next compared the patterns of histone protein during the sporulation process by SDS–polyacrylamide gel electrophoresis. Fig.2 is a representation of

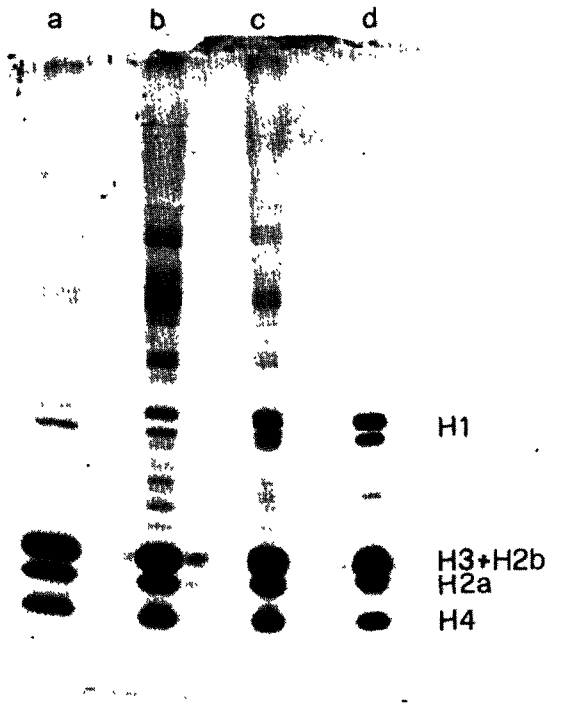

Fig.2. Analysis of histones by gel-electrophoresis: (a) histones extracted from chromatin of cells incubated in sporulation medium for 6 h; (b,c) histones from vegetatively growing yeast cells, alone (b) and prepared from a mixture of yeast nuclei and rat liver nuclei (c); (d) histones isolated from rat liver nuclei according to the procedure for the preparation of yeast histones are shown for comparison. About equal amounts of total proteins were applied to each slot. Gels were stained with 0.25% Coomassie blue in 7% acetic acid in 50% methanol.

typical histone patterns obtained from vegetatively growing yeast cells (b) and cells which had been incubated for 6 h in sporulation medium before histone preparation (a). There is no difference in the patterns of conservative histones (H2a, H2b, H3, H4) between the two preparations. Comparison with the pattern of rat liver histones (fig.2d) reveals the existence of two faint protein bands with a mobility only slightly different from the rat H1 bands. Histone preparations from both, vegetatively growing and sporulating yeast cells, contain these proteins – which, if anything, are somewhat more prominent in growing than in sporulating cells. Hence, our hope to eventually obtain more convincing evidence for the existence of yeast H1 histone by analysing proteins from sporulating cells (see section 1) was not fulfilled. Other laboratories had claimed the finding of a yeast histone H1 protein, banding in SDS–polyacrylamide gel at a position different from that of vertebrate H1 [10–12] and from our bands in the H1 region. As histone H1 is known to be particularly sensitive to proteases, it might have been partly lost during our careful preparation of chromatin, in spite of the fact that all our buffers contained various protease inhibitors. Hence, in a control experiment we added nuclei from rat liver to the yeast protoplasts during lysis in the Ficoll buffer. At the end of the preparation procedure the rat histone H1 bands were clearly detectable in the electrophoresis pattern (fig.2c). The possibility that the histone H1-like proteins in [10–12], which were only found by using preparation methods different from our own procedure and which appear to differ at least in electrophoretic properties from laboratory to laboratory, are in fact not related to H1, can likewise not be excluded so far. In this regard it is interesting to note that the two weak bands incorporate lysine poorly (fig.3) which confirms observations in [3] and does not support the idea that these bands represent H1 histones.

We also determined the distribution of [^{3}H]lysine label incorporated into the 4 inner histones by scanning autoradiograms of histone patterns. Fig.3 shows 3 examples of scans of autoradiograms of yeast histones which were separated electrophoretically. By determining the amount of label in histone fractions (peaks of H3 + H2b, H2a and H4, respectively) we conclude that none of the histone classes was preferentially synthesized at any time point during the sporulation process, as the ratios of radioactivity were found nearly identical at different times.

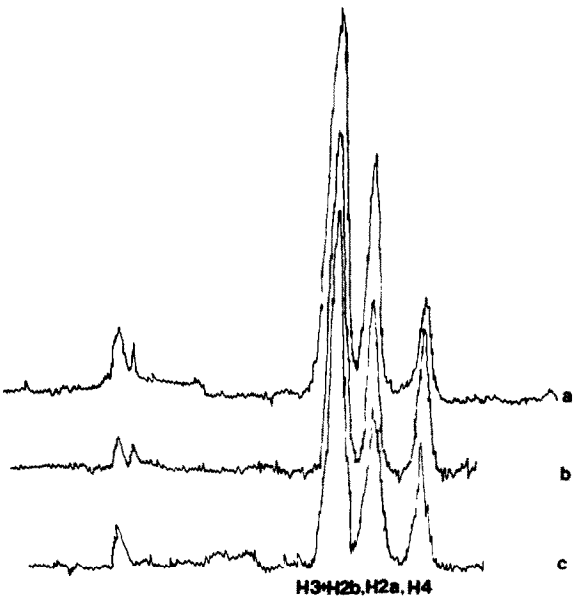

Fig.3. Electropherogrammes of labeled histones isolated during the sporulation process. Labeled histones were separated on 18% SDS–polyacrylamide slab gels (6 h at 50 mA) and fluorographed as in the text. Shown are the scans of the fluorograms: (a) before; (b) 3 h; (c) 6 h after transfer to sporulation medium.

These results strongly suggest another case of uncoupled histone synthesis during cell commitment, described while this work was in progress [13]: Friend cells, in vitro induced to terminal differentiation by butyrate, also start histone synthesis in advance of the somewhat delayed DNA replication during the last cell cycle before hemoglobin synthesis becomes detectable. Thus a change in the timing of DNA and histone synthesis might be a common feature to processes of cellular reprogramming, of which yeast sporulation might be a phylogenetically very old example.

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