

Identification and isolation of core histones from *Schizosaccharomyces pombe*

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Core histones have been isolated from *Schizosaccharomyces pombe* and compared electrophoretically to core histones from *Saccharomyces cerevisiae* and rat liver. The molecular masses of all cognate histones examined were found to be very similar as determined by SDS gel electrophoresis. Histones H3, H2A and H2B from *Sch. pombe* migrated almost identically to their respective counterparts from *S. cerevisiae* as determined by acid/urea gel electrophoresis. Two-dimensional gel electrophoresis with a Triton X-100 acid/urea gel in the first dimension followed by an SDS gel in the second dimension was used to separate *Sch. pombe* histones from contaminating ribosomal proteins.

Core histone; Histone isolation; Gel electrophoresis; (*S. cerevisiae*; *Sch. pombe*)

1. INTRODUCTION

In 1971 the presence of a single histone protein from *Schizosaccharomyces pombe* of molecular mass 40 kDa was reported [1]. The *Sch. pombe* nuclear preparation obtained by the above method contained a high RNA:DNA ratio [2]. Due to this factor and to the immunological cross-reactivity between the proposed histone and a ribosomal protein, the possibility cannot be excluded that the proposed histone protein [1,3] was of ribosomal origin.

During mitosis in yeasts, the chromosomes are separated by spindle fibers and the nuclear membrane does not break down [4]. Histones have previously been identified in other yeasts, most notably *Saccharomyces cerevisiae* [5,6].

We report here the identification of histones from *Sch. pombe*. *Sch. pombe* appears to contain a full complement of core histones, similar in molecular mass to cognate histones from *S.*

cerevisiae. These findings are consistent with the recent isolation and cloning of *Sch. pombe* histone genes using as probes histone genes from *S. cerevisiae* and *Drosophila* [7,8].

2. MATERIALS AND METHODS

2.1. *Organisms and growth conditions*

Sch. pombe 972 h⁺ and *S. cerevisiae* 10275 α were obtained from the National Collection of Yeast Cultures and the American Type Culture Collection, respectively. These yeasts were grown in YEL (0.5% yeast extract and 3.0% glucose) at 37°C without shaking.

2.2. *Isolation of yeast chromatin acid-extractable proteins*

Yeast chromatin was isolated essentially as described by Sommer [6] with minor modifications. Buffer A contained 50 mM Tris instead of 25 mM, only one washing of the chromatin pellet was carried out after centrifugation through 1.7 M sucrose, and 1.0 mM phenylmethylsulfonyl fluoride was used instead of 0.4 mM. The above method employs glass beads to disintegrate yeast

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cells by hand shaking. Chromatin was then isolated from the whole-cell homogenate by differential centrifugation and pelleting through 1.7 M sucrose.

Histones were extracted by suspending the chromatin pellets in 0.5 N HCl for 2–24 h. Unsolubilized material was pelleted by centrifugation and the acid-extractable proteins were precipitated with 25% trichloroacetic acid at 4°C for 1 h. The precipitate was pelleted by centrifugation and washed once with acetone containing 0.05% HCl and twice with acetone without HCl. All acetone washings were done at –20°C. The washed precipitates were dried in vacuo and resuspended in sample buffers for either acid/urea [9] or SDS gel electrophoresis. The SDS sample buffer contained 100 mM Tris-HCl (pH 6.8), 2.3% SDS, 2.3 M urea, 5 mM EDTA, 2.3% glycerol, 2.3% 2-mercaptoethanol and 0.002% bromophenol blue.

2.3. Isolation of rat liver histones and *Sch. pombe* ribosomal proteins

Rat liver nuclei were isolated by pelleting through dense sucrose essentially as described by Blobel and Potter [10] and chromatin was purified as described by Stankiewicz et al. [11]. *Sch. pombe* ribosomes were isolated by the method of Codrington and Fluri [12]. Rat liver histones and yeast ribosomal proteins were extracted, precipitated, and resuspended as described above.

2.4. One-dimensional gel electrophoresis

Acid/urea gel electrophoresis was performed as described by Panyim and Chalkey [9]. These gels contained 2.5 M urea. All SDS gels contained 15% acrylamide and were prepared and run as described by Laemmli [13]. Gels were stained with Coomassie brilliant blue R-250.

2.5. Two-dimensional gel electrophoresis

Proteins to be analyzed on two-dimensional gels were dissolved in 6 M urea, 5% 2-mercaptoethanol, 5.4% glacial acetic acid, 16.5% glycerol, 0.3% methyl green and 0.4% Triton X-100. The first dimension was an acid/urea slab gel (0.8 mm thick) as described above except that it contained 6 M urea and 0.4% Triton X-100. The second dimension was an SDS slab gel (1.5 mm thick) as above. After staining with Coomassie blue and

destaining, the lanes of the first dimension were cut into strips and soaked for 30 min in deionized water. The gel strips were then placed in an SDS sample buffer for 1 h prior to electrophoresis in the second dimension. The SDS sample buffer was as described [14]. Rat liver histone markers for the second dimension were electrophoresed on a first dimension gel until all of the tracking dye penetrated the gel. The small gel containing the rat liver histones was then treated identically to the yeast proteins to be analyzed in the second dimension.

3. RESULTS

3.1. SDS gel electrophoresis of acid-extractable proteins

SDS gel electrophoresis was performed on acid extracts of chromatin from *Sch. pombe*, *S. cerevisiae*, and rat liver as well as *Sch. pombe* ribosomal proteins (fig.1). Bands were clearly visible in the *Sch. pombe* chromatin lane which matched up fairly closely with the core histone bands of the other two organisms shown here. Since the order of migration of *S. cerevisiae* and rat liver histones was the same on SDS gels, the bands in lane 1 were presumed to be core histones as labeled in fig.1.

A comparison of lanes 1 and 5 in fig.1 indicated that, while there was a considerable amount of ribosomal protein contamination in the *Sch. pombe* chromatin extract, protein other than that of ribosomal origin was present. One demonstration of this point is that no ribosomal protein migrated similarly to histone H4 in this gel system.

3.2. Acid/urea gel electrophoresis of acid-extractable proteins

Histones from *S. cerevisiae* have a different order of migration on acid/urea gels containing 2.5 M urea than those from higher eukaryotes [6]. *S. cerevisiae* histones migrate from the positive to negative pole on an acid/urea gel in the order H2B, H2A, H3 and H4. Histones from higher eukaryotes migrate in the same order on both acid/urea and SDS gels. Acid/urea gel electrophoresis (fig.2) was performed on preparations identical to those used in fig.1. With the exception of H4, there was a close correspondence in the banding pattern of the *Sch. pombe* and *S.*

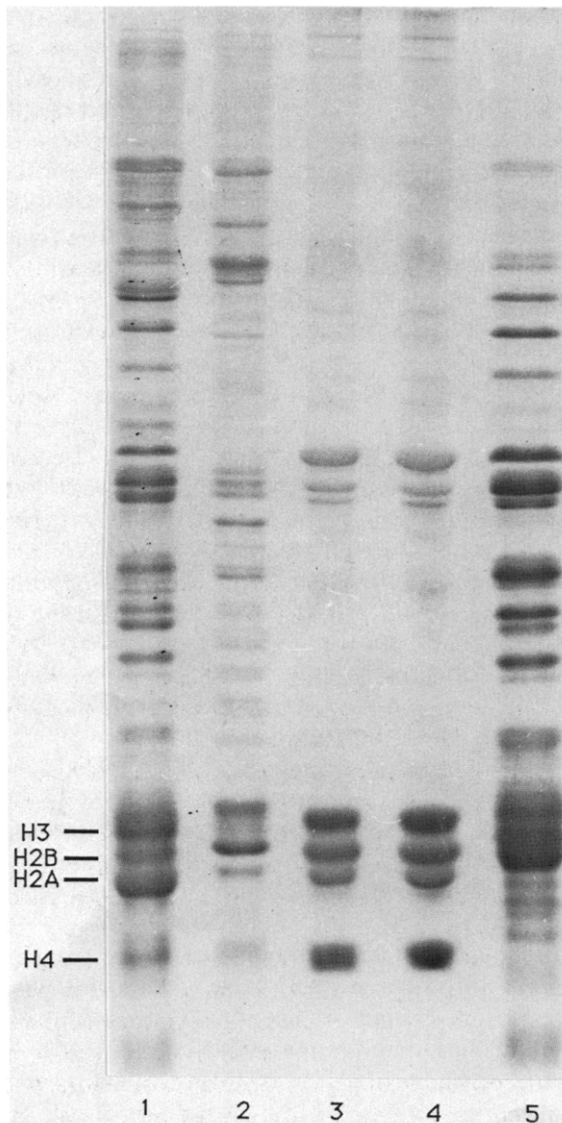


Fig.1. SDS gel electrophoresis of histones from yeasts and rat liver. Lanes: 1, an acid extract of *Sch. pombe* chromatin; 2, a similar preparation of *S. cerevisiae* chromatin; 3,4, rat liver histones; 5, an acid extract of *Sch. pombe* ribosomes.

cerevisiae chromatin acid extracts in the core histone range. None of the ribosomal proteins present migrated similarly to the yeast histones H2A and H4 in the acid/urea gel. While some ribosomal proteins migrated similarly to the yeast histones H2B and H3, the intensity of the bands strongly suggested that most of this protein is not

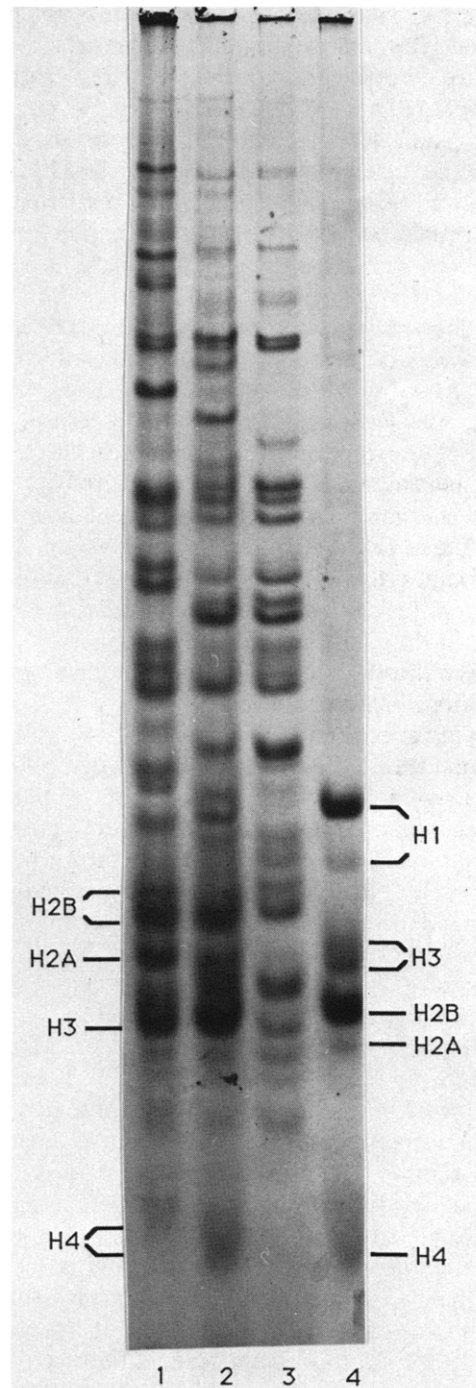


Fig.2. Acid/urea gel electrophoresis of histones and ribosomal proteins. Samples are identical to those in fig.1. Lanes: 1, an acid extract of *S. cerevisiae* chromatin; 2, a similar preparation from *Sch. pombe*; 3, *Sch. pombe* ribosomal protein; 4, rat liver histones.

ribosomal. *S. cerevisiae* contains two forms of histone H2B that are coded for by different genes and are of similar molecular mass, but of slightly different charge [15]. As can be seen in fig.2, two H2B bands in the *S. cerevisiae* lane were resolved in this gel system. Only one H2B gene has been found in *Sch. pombe* [8] and only one band corresponding to H2B appeared to be present in the *Sch. pombe* lane of fig.2.

3.3. Two-dimensional gel electrophoresis of *Sch. pombe* chromatin acid-extractable proteins

The two-dimensional gel electrophoresis performed here employed a Triton X-100 acid/urea gel in the first dimension and an SDS gel in the second. A similar gel system has previously been reported to be capable of separating histones from ribosomal proteins [14]. Triton X-100 binds to the hydrophobic regions of histones and slows them down in the first dimension allowing resolution in the second dimension. The result is that most of the ribosomal proteins migrate along a diagonal while histones migrate in the same region off the diagonal. Fig.3 shows a two-dimensional gel of an *Sch. pombe* chromatin acid extract showing intense bands off the diagonal which migrated in a

similar fashion to histones from *S. cerevisiae* (not shown). In the second dimension, the *Sch. pombe* histone proteins migrated almost identically to the rat liver histone markers on the left of the gel.

4. DISCUSSION

The present data demonstrate that *Sch. pombe* contains a full complement of core histones that migrate similarly to those from *S. cerevisiae* on acid/urea and SDS gels. Because of the extremely low DNA content of yeast cells compared to higher eukaryotes [16,17], most procedures for the isolation of histones from *S. cerevisiae* begin with spheroplasts from which intact nuclei are obtained. However, due to differences in the cell wall structure [18], most methods used to generate spheroplasts from *S. cerevisiae* are not effective for *Sch. pombe*. While spheroplasts of *Sch. pombe* can be prepared [19], we found it impossible to isolate histones from nuclei employing this method. This was presumably due to the presence of proteases in the enzyme mixture used to generate the spheroplasts.

Since the methods employed here involved the isolation of chromatin from a whole-cell homogenate, the resulting protein extract inevitably contained a significant amount of ribosomal contamination. Since SDS gel electrophoresis is not an effective method for the separation of histones from yeast ribosomal proteins [3], a number of different gel systems were used. SDS gel electrophoresis separates proteins mainly by molecular mass [13], while acid/urea gels separate through a combination of charge and molecular mass [9]. The two-dimensional gel system combines these separation properties and the ability of Triton X-100 to bind to histone proteins, thereby inhibiting their mobility in the first dimension [14].

Recently, genes coding for the core histones of *Sch. pombe* have been isolated [7,8]. The predicted amino acid composition suggests that these histones are similar in size and charge to their *S. cerevisiae* counterparts. The data presented here are consistent with these findings.

We have shown that *Sch. pombe* contains a full complement of core histones. These proteins migrate similarly to both rat liver and *S. cerevisiae* histones on SDS gels and similarly to *S. cerevisiae*

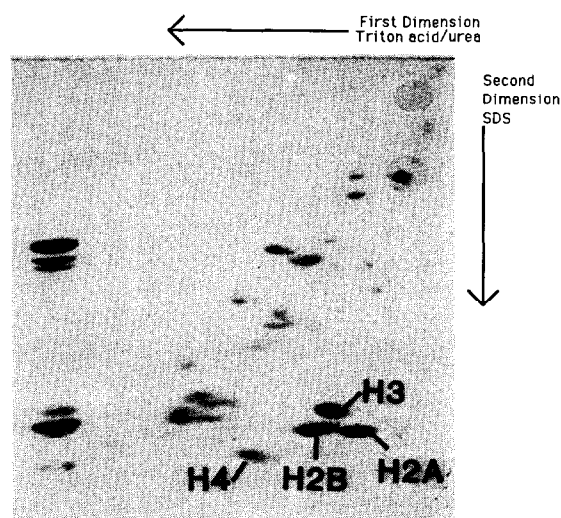


Fig.3. Two-dimensional gel electrophoresis of an *Sch. pombe* chromatin acid extract. *Sch. pombe* proteins are on the right. Rat liver histone markers are in the vertical lane on the left.

histones on acid/urea gels. The *Sch. pombe* histones also migrate similarly to those of *S. cerevisiae* on the two-dimensional gel system used here. This gel system is effective in separating histones from ribosomal protein contamination.

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