Heterologous expression of human H1 histones in yeast

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Abstract The complete set of seven human H1 histone subtype genes was heterologously expressed in yeast. Since *Saccharomyces cerevisiae* lacks standard histone H1 we could isolate each recombinantly expressed human H1 subtype in pure form without contamination by endogenous H1 histones. For isolation of the H1 histones in this expression system no tagging was needed and the isoforms could be extracted with the authentic primary structure by a single extraction step with 5% (0.74 M) perchloric acid. The isolated H1 histone proteins were used to assign the subtype genes to the corresponding protein spots or peaks after two-dimensional gel electrophoresis and capillary zone electrophoresis, respectively. This allowed us to correlate transcriptional data with protein data, which was barely possible until now.

Key words: H1 histone; Heterologous expression; *Saccharomyces cerevisiae*; Two-dimensional gel electrophoresis; Capillary zone electrophoresis

1. Introduction

Histones are the basic proteins responsible for the nucleosomal organization of the chromosomal fiber in eukaryotes. The core histones H2A, H2B, H3, and H4 form the core structure of the nucleosome, and the linker histone H1 is involved in sealing two rounds of DNA at the nucleosomal core (reviewed by Cole [1]). H1 histones are also involved in the formation of higher order structures of chromatin [2] and contribute as basal repressors to the regulation of gene activity [3]. With the exception of H4 each class of these nuclear proteins consists of several subtypes which are encoded by different genes. These are organized in clusters, which may be either arranged as tandem repeats of the five histone genes as in the genomes of *Drosophila melanogaster* or of sea urchins, or as apparently random groups of histone genes as in mammals [4]. Non-tandemly repeated arrangements of histone genes in the histone gene clusters of chicken, mouse and man have previously been reported. We have isolated the genes for all human H1 histones [5–9] and have shown that six of the seven human H1 genes are clustered on the short arm of chromosome 6, whereas the gene for the replacement variant H1° is solitarily located on chromosome 22 [10].

Ohe et al. [11,12] have sequenced four H1 proteins isolated from human spleen and separated by reversed phase HPLC, and Parseghian et al. [13] have partially sequenced four H1 proteins isolated from human placenta. The sequences are identical with the deduced amino acid sequences of the isolated genes (for assignment, see Drabent et al. [14]).

In contrast to higher eukaryotes, *Saccharomyces cerevisiae* seemed to have no linker histone [15]. On sequencing the entire yeast genome a sequence of an open reading frame (ORF) with similarity to the globular domain of linker histones has been identified, and the gene has been interpreted as the H1 histone gene (Hho1) of yeast [16]. Deletion of the gene in *Saccharomyces cerevisiae* had no significant phenotypic effect [17,18], except that the expression of β-galactosidase from a cytochrome *c*-lacZ reporter plasmid was altered [19]. Although recombinantly expressed Hho1p binds in vitro to the linker DNA of reconstituted core dinucleosomes [18], until now no specific linker histone in vivo function has been ascribed to this protein, and it cannot be excluded that the H1-like protein is rather a gene specific transcription factor, like the linker histone structural homologue HNF3-γ [20], than a standard linker histone.

Since extraction of untransformed yeast cells with 5% perchloric acid (PCA) does not yield proteins in the size range of H1 histones, we considered the yeast *Saccharomyces cerevisiae* as an expression system suitable for the isolation of pure human H1 histone subtype proteins. The prokaryotic expression system of *E. coli* turned out to be unsuitable to express linker histones, since heterologous expression of the arginine rich chicken linker histone H5 in *E. coli* failed to yield full length histone proteins [21]. Similarly, efforts to express histone H5 of *Xenopus laevis* in the yeast *Saccharomyces cerevisiae* also did not result in full length products [22].

Here we describe the expression of the complete set of seven human H1 histones in the yeast *Saccharomyces cerevisiae* and their application to calibrate the two-dimensional gel electrophoresis system as well as the capillary zone electrophoresis for the analysis of histone H1 subtype patterns.

2. Materials and methods

2.1. Expression plasmids

The human H1 histone genes were inserted into the *Sal*I/*Bam*HI site of the yeast expression vector YEp51 [23]. Molecular cloning was done as described in Sambrook et al. [24]. The plasmid contains the GAL10 promoter, so that expression of H1 histones could be induced by addition of galactose to the growth medium. The cloning strategy is shown in Fig. 1. The resulting plasmids were transformed into the yeast strain *S. cerevisiae* ENY.WA-4D [25] by the lithium acetate method [26].

2.2. Yeast strain and media

The yeast strain ENY.WA-4D (MATa leu 2-3,112 ura3-52) was used to express the histone genes.

Transformed yeast cells were grown in SCGL-Leu medium: 2%

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Abbreviations: APS, ammonium persulfate; PCA, perchloric acid; TCA, trichloroacetic acid; CZE, capillary zone electrophoresis; RPMI, Roswell Park Memorial Institute medium; HPMC, hydroxypropylmethylcellulose; PBS, phosphate buffered saline; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; AUT gel, acid-urea-triton electrophoresis gel; TEMED, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine

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lactic acid; 3% (v/v) glycerol; 0.7% yeast nitrogen base without amino acids; 0.1% glucose; 10 ml 10× amino acid mix/100 ml (10× amino acid mix contained per liter: 200 mg each of adenine, uracil, histidine, proline, tryptophan, arginine, and methionine; 300 mg of isoleucine, tyrosine and lysine; 500 mg of phenylalanine; 1 g of glutamic acid, aspartic acid, valine, threonine and serine). SCD-Leu medium contained: 4% glucose; 0.7% yeast nitrogen base without amino acids and 10 ml 10× amino acid mix/100 ml. In SCGal-Leu medium glucose was substituted by galactose.

YPGLA medium contained: 1% yeast extract; 2% peptone; 0.2% glucose; 3% (v/v) glycerol; 2% lactic acid; 2 mg adenine/100 ml.

2.3. Expression and purification of H1° in yeast

For expression of human H1 histones 5 ml SCGL-Leu medium was inoculated with 100 μl of a yeast preculture (ENY.WA-4D transformed with the H1 construct) and was grown at 30°C overnight. One ml of this overnight culture was transferred into 200 ml of SCGL-Leu medium and incubated under agitation at 30°C until an OD_{600} of 1.0 was reached. Then 200 ml of YPGLA medium was added, the culture was incubated for 3 h, and expression of H1 histone protein was induced by addition of 8 g galactose per 400 ml. Following incubation for 6 h (OD_{600}: 1.2), the yeast cells were harvested by centrifugation at 2900×*g* for 10 min in a Sorvall RC2-B centrifuge. The pellet was suspended in 30 ml H_2O and recentrifuged. The pellet was resuspended in 20 ml PTB (1 M sorbitol; 4.25 mM KH_2PO_4; 45.75 mM K_2HPO_4) with 20 mM dithiothreitol. Thirty min after incubation at 37°C the suspension was centrifuged for 5 min. The pellet was resuspended in 15 ml DB (1 M sorbitol; 1 mM EDTA) with 10 mg zymolyase T20 (Medac, Hamburg, Germany) to digest the cell wall. After 30 min at 37°C the resulting spheroplasts were centrifuged for 10 min at 4300×*g*, washed with DB, and an equal volume of glass beads was added to the pellet. After addition of 1 ml of 0.75 M (5%) $HClO_4$, the mixture of cells and glass beads was thoroughly vortexed and incubated for 30 min on ice. After centrifugation for 10 min at 12 000×*g*, the supernatant was collected, and the pellet was extracted once again using the procedure described above. The pooled supernatants were then incubated with TCA in a final concentration of 20% or alternatively with a 9-fold volume of ice-cold acetone overnight at −20°C. After centrifugation at 13 000×*g* the precipitated H1 histones were washed with acetone. The pellet was solubilized in 10 mM HCl by repeated stirring and incubation on ice.

For CZE separation the H1 histones had to be further purified. Therefore, the H1 histones were precipitated by saturating the 10 mM HCl solution with $(NH_4)_2SO_4$ (final concentration approx. 90% w/v) followed by incubation for 1 h on ice. The samples were centrifuged (20 min, 13 000×*g*) and the pellets were resolubilized in 10 mM HCl. A third precipitation step followed (addition of TCA to a final concentration of 20% w/v) to remove traces of ammonium sulfate. After incubation for 1 h on ice, samples were centrifuged (20 min, 13 000×*g*) and the pellets were resolubilized in 10 mM HCl and analyzed for purity by SDS-polyacrylamide gel electrophoresis.

2.4. Isolation of H1 histones from HeLa cells

The human tumor cell line HeLa S3 was cultured in RPMI 1640 supplemented with 10% (v/v) fetal calf serum. The cells were grown adherently at 37°C (5% CO_2) to a density of 5×10^4 cells/cm^2 and scraped off using a cell scraper. The cells ($1–2\times10^8$) were washed once with phosphate-buffered saline (PBS). Then 0.5 ml of 0.75 M $HClO_4$ was added to the cell pellets, cells were lysed and histones were extracted by incubation for 1 h at RT under continuous stirring. Samples were centrifuged and the supernatant was stored while the pellet was reextracted once with 0.5 ml of 0.75 M $HClO_4$. Supernatants were collected and centrifuged again. The acid-soluble proteins were precipitated by addition of 9 volumes of ice-cold acetone and overnight incubation at −20°C, or alternatively, the H1 histones were precipitated by TCA at a final concentration of 20% (v/v) for one hour on ice.

After centrifugation (30 min, 13 000×*g*), pellets were washed twice with cold acetone and vacuum-dried. The histones were then solubilized in 10 mM HCl and subjected to analysis by CZE.

2.5. Two dimensional gel electrophoresis

Two dimensional gel electrophoresis was done using minigels (100×100 mm, 1 mm thickness). First dimension: acid-urea-Triton X-100 (AUT) polyacrylamide gels. AUT-gels consisted of 13.5% acrylamide (acrylamide/bisacrylamide: 30:0.5) in the separating gel and 5% in the stacking gel, 1 M acetic acid, 6.25 M urea, pH 3.5, 0.2% Triton X-100, ammonium persulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED). The electrophoresis buffer consisted of 0.5 M acetic acid and 0.2 M glycine, pH 3.5.

An equal volume of sample buffer (0.1% Pyronin Y and 1% β-mercaptoethanol in 6 M urea) was added to the samples. The gels were prerun for 1 h at max. 20 mA/gel. Electrophoresis was performed at max. 20 mA, and continued for 90 min after the dye front had reached the end of the gel.

Gels were stained in 0.1% amido black, 40% methanol, 10% acetic acid, 50% distilled water (v/v). Destaining was performed in 40% methanol, 10% acetic acid, 50% water, cut into stripes and equilibrated before separation on SDS-PAGE was performed. The first equilibration step was carried out in 50 mM acetic acid, 0.5% SDS for 30 min; the second in 60 mM Tris-HCl, pH 6.8, 0.2% SDS for 20 min.

In the second dimension we used SDS-PAGE according to Laemmli [27], using 15% acrylamide in the separating and 5% in the stacking gels. Gel stripes were fixed between the glass plates and embedded in the stacking gel. Electrophoresis was performed at 20 mA until the dye front was eluted. The gels were stained and destained as described above.

2.6. Capillary zone electrophoresis (CZE) of H1 histones

Capillary zone electrophoresis (CZE) was performed on a Perkin Elmer/Applied Biosystems 270A-HT system. The method of Lindner et al. [28,29] was optimized for separation of human H1 histone subtype proteins (Kratzmeier et al., submitted). Briefly: separations were performed under the following conditions: temperature 30°C, voltage 12 kV, current approx. 35 μA, separation time 25 min, sample injection by pressure, injection time 2 s, sample protein concentration about 0.5 mg/ml, absorbance detection at 200 nm. The capillary type was fused silica, length 72 cm, ID 50 μm. Separation buffer was 30 mM H_3PO_4, 60 mM $HClO_4$, 0.02% hydroxypropyl-methylcellulose (HPMC), pH 2.0, adjusted with triethylamine. Absorbance data were recorded and analyzed by a PC-based Kontron Integration System.

3. Results

3.1. Expression plasmids

For expression of the human H1 histone subtype proteins the coding region and short portions of the flanking sequences of the seven human H1 histone genes were inserted into the yeast expression vector YEp51 [23] containing the inducible promoter of the yeast GAL10 gene. The termination sequence of the yeast ADH1 gene was fused to the 3′ end of some of the constructs (see below) for a defined transcriptional termination in order to achieve higher expression (Fig. 1). The inducible expression system was chosen, since we could not exclude a growth inhibiting or lethal effect of the expressed heterologous H1 histones on the transformed yeast cells. Growth inhibition may have resulted in a very low transformation rate or even in lack of transformants. Therefore we first plated the transformation mixture on SCD-Leu plates (repressible conditions) to select only for transformation. The subsequent cultivation of the transformants under inducing conditions (SCGal-Leu medium) did not result in growth repression. Another advantage of the GAL expression system is the high expression level of genes under the control of the GAL10 promoter, since this promoter is one of the strongest promoters identified in yeast. The lengths of the 5′-UTR and the 3′-UTR, respectively, of the fragments of the human histone H1 genes were different, since the sequences of the genes differed in this region and therefore no common restriction sites could be used. Thus, the fragments were flanked by different ends. The sticky ends of the fragments were turned into

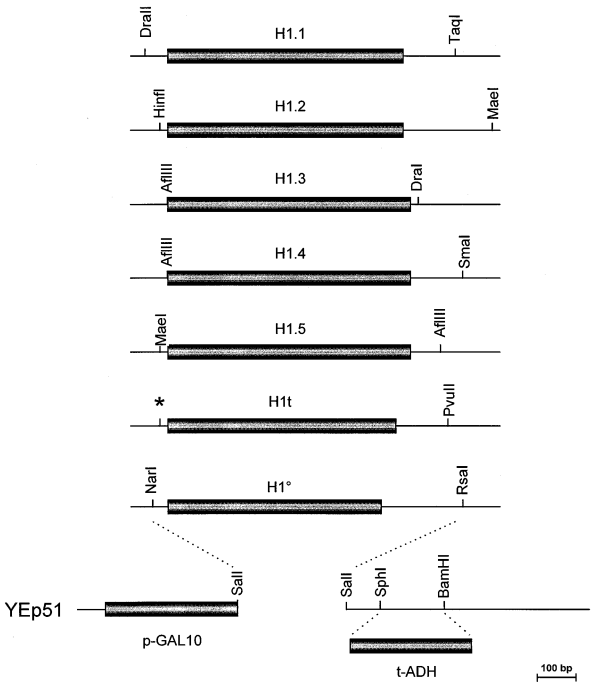

Fig. 1. Construction of the expression plasmids. The indicated gene fragments of the human histone H1 genes were isolated from plasmids with inserts of genomic DNA containing the entire H1 coding sequences. The recombinant plasmids were cleaved with the indicated restriction endonucleases, the isolated fragments were filled in with Klenow polymerase and ligated to the *Sal*I sites (also blunt ended by filling in) of the yeast expression vector YEp51. The resulting plasmids containing the genes for H1.1, H1.2, H1.4 and H1° were cleaved with *Sph*I, the sticky ends were filled in and the linearized plasmids were then cleaved with *Bam*HI. The 0.4-kb *Hin*dIII(fill in)/*Bam*HI fragment of the 3′-UTR of the yeast ADH1 gene was ligated to these linearized plasmids. H1.3, H1.5 and H1t clones remained without addition of the 3′-UTR. For the construction of the H1t expression plasmid, an artifical *Sal*I site at the 5′ end was generated by a double stranded oligonucleotide. This site is indicated by an asterisk. p-GAL10 indicates the promoter region from the yeast GAL10 gene and t-ADH indicates the terminator region of the yeast alcohol dehydrogenase gene (ADH1). The coding regions of the genes are shown as filled rectangles. The length of the 3′- and 5′-UTR of the individual gene fragments can be estimated using the scale.

blunt ends by a fill-in reaction with Klenow polymerase. The resulting fragments were cloned into the blunt ended *Sal*I site of the expression vector YEp51. The restriction sites used and the portions of the 5′-UTR and 3′-UTR regions are indicated in Fig. 1. The only exception from this cloning strategy was the H1t gene. Since there were no suitable restriction sites within the 5′-UTR of the H1t gene, we first isolated a 733-bp *Pst*I/*Pvu*II gene fragment that ends 21 nucleotides downstream of the translational start side. In a second step a double stranded oligonucleotide containing the missing nucleotides with a *Sal*I restriction site on the one end and a *Pst*I site on the other end was ligated in front of the *Pst*I/*Pvu*II gene fragment. The resulting fragment was cloned into the blunt ended *Bam*HI site and the *Sal*I site of YEp51 [30]. Since the expression rate of some of the constructs was very low we cloned the 3′ termination sequence of the yeast ADH1 gene downstream of the H1 histone genes within the expression vectors for H1.1, H1.2, H1.4 and H1°. The ADH1 terminator fragment was isolated by linearizing the plasmid pAAH5 [31] with the restriction enzyme *Hin*dIII. The sticky ends of the *Hin*dIII cleavage site were filled in by Klenow polymerase. Subsequent cleavage of the linearized vector DNA with *Bam*HI resulted in a 0.4-kb fragment that was cloned into the *Bam*HI and the blunt ended *Sph*I site of the respective YEp51 derivatives.

3.2. Purification of recombinant H1 histones

To yield high amounts of recombinant human H1 histones, we developed a combination of the enzymatical and mechanical method for yeast cell breakage. With the two methods combined almost all cells were lysed even when harvested in the late log/stationary phase with a high cell density (OD_{600}: 4.0). The cell walls were first removed by zymolyase digestion and then in a second step the resulting spheroplasts were lysed and the remaining zymolyase resistant cells were broken up by blending with glass beads directly in 5% PCA. Extraction of whole cell extracts with 5% PCA resulted in a selective enrichment of H1 proteins because of their high acid solubility [32]. After centrifugation of the cell debris and the acid insoluble cell material, the H1 histones were collected out of the supernatant by precipitation with 20% TCA or alternatively by addition of 9 volumes of ice-cold acetone. This simple and rapid purification method yielded human H1 histones in high amounts and of relatively high purity. The only contaminating proteins detected by amido black staining of SDS-PAGE gels were in the range of 10 kDa. We conclude that these proteins are endogenous yeast proteins and no degradation products of the expressed recombinant H1 histones, since the amount of these contaminating proteins was independent from the expression rate of the recombinant H1 histone. Precipitation of the perchloric acid soluble H1 histones with acetone instead of TCA reduced the relative amount of contaminating proteins but also lowered the yield of recombinant H1 histone. The direct breaking of cells and spheroplasts in 5% PCA prevented proteolytic degradation of the recombinant H1 histones during preparation. The yield of recombinant human H1 proteins varied between about 10 μg (H1t) and 2 mg (H1°) from a 4-l culture. The isolated histone H1 proteins were analyzed by SDS-PAGE (Fig. 2). The relative migration was in accordance with the theoretical molecular weight of the

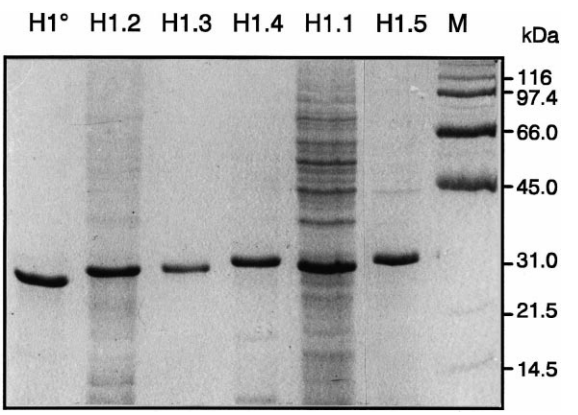

Fig. 2. SDS-PAGE of the isolated H1 subtype proteins. The H1 histones were isolated by 5% PCA extraction and separated on a 12.5% SDS-PAGE gel. Protein bands were visualized by staining with amido black. Two μg of histone H1°, H1.2, H1.4, H1.1, H1.5 and 0.5 μg of H1.3 were loaded on the gel. The protein concentration was determined by the Bio-Rad Protein Assay (Bio-Rad, Richmond, CA, USA) using a standard curve based on calf thymus H1. M: protein size markers.

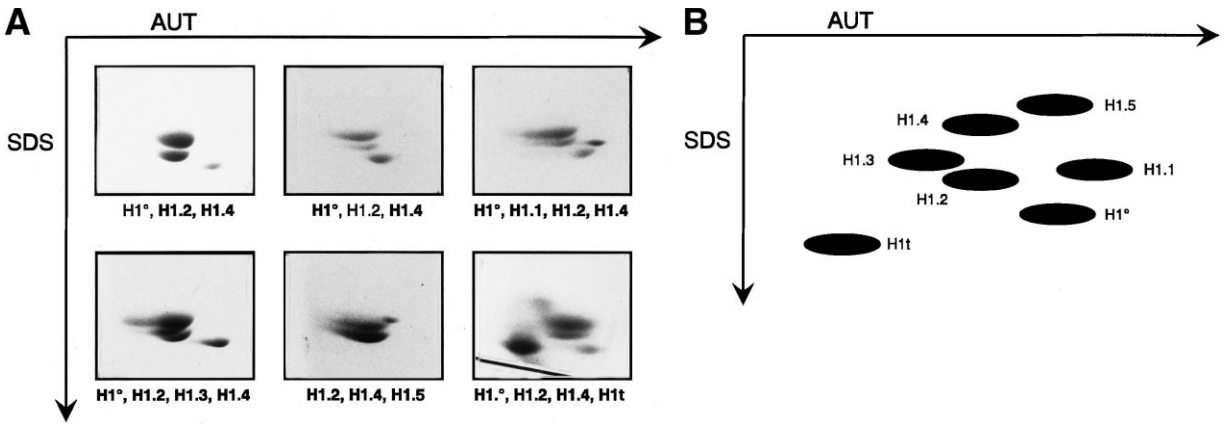

Fig. 3. Two-dimensional gel electrophoresis of mixtures of recombinant H1 subtypes. A: Electropherogram of the two-dimensional gel electrophoresis. The H1 histones were separated in the first dimension by AUT gel electrophoresis (13.5% acrylamide, 1 M acetic acid, 6.25 M urea, pH 3.5, 0.2% Triton X-100). Separation in the second dimension followed by SDS-PAGE (15% acrylamide). Running conditions are described in Section 2. Two μg of each of the indicated H1 subtypes were loaded on the gel. The subtype composition of the mixtures is listed in bold letters below the electropherograms. Subtypes listed in normal letters were added at a quantity of just 0.5 μg (first two pictures). H1t was additionally purified by ammonium sulfate precipitation as described above (purification before CZE, see Section 2). B: Schematic map of the electropherogram of all human H1 subtype proteins. The relative positions of the H1 subtypes were estimated from the electropherograms shown in A.

subtypes. The H1 subtypes isolated by PCA extraction from both the yeast transformants and human cell cultures are not phosphorylated [30]. Therefore it is possible to assign the H1 subtypes isolated by PCA extraction from culture cells directly to the respective recombinant H1 subtypes and furthermore to the subtype genes.

3.3. 2D-polyacrylamide gel electrophoresis of H1 subtypes

Recombinant H1 histone proteins were used to determine the relative position of the spots of the individual H1 subtypes in a two-dimensional gel electrophoresis system optimized for H1 histones and adapted to minigels (100×100 mm, 1 mm thickness) [30]. The H1 proteins were separated in the first dimension on acid-urea-Triton (AUT) gels and in the second dimension by SDS-PAGE. The relative positions of the individual H1 subtypes after electrophoresis in this gel system were assigned by mixing experiments with the recombinant H1 proteins. The spots of the recombinant H1°, H1.2 and H1.4 subtypes were determined by mixing different relative amounts of each H1 subtype (first two pictures of Fig. 3A). The spots of the other subtypes were determined by successively adding them to the mixture of H1°, H1.2 and H1.4 (Fig. 3A). Using these data we generated a map of the relative positions of the spots of the human H1 histone subtypes after

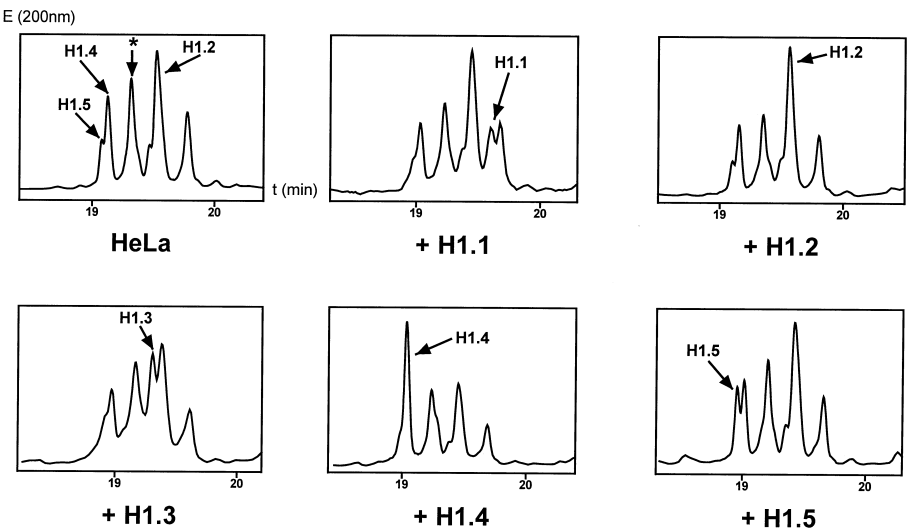

Fig. 4. CZE electropherograms of recombinant human histone H1 subtypes mixed with HeLa histone H1 preparations. HeLa cells were harvested and H1 histones were prepared by PCA extraction as described in Section 2. H1 histones were then solubilized in 10 mM HCl and subjected to CZE using a fused-silica capillary with a total length of 72 cm and an inner diameter of 50 μm. Absorbance data (E) were continuously recorded. The electropherograms display only the absorbance curves between 18 and 21 min after injection. To identify the peaks of the individual subtypes within the pattern of H1 histones, each of the human H1 subtypes was mixed with an aliquot of the histone H1 preparation from HeLa cells. Maximal absorbance at 200 nm was between 0.007 and 0.018. The peak marked with an asterisk represents the High Mobility Group protein HMG17 (Kratzmeier et al., unpublished). The mixing experiments with recombinant H1° and H1t (both not present in HeLa cells) are omitted in the figure, since the elution times of these subtypes were outside the time period shown (15 min for H1° and 23 min for H1t).

electrophoresis in the 2D gel electrophoresis system described above (Fig. 3B). This assignment of the H1 subtypes to the spots on the two-dimensional gels allows for the first time a rapid and simple analysis of histone subtype gene expression in different cells and under different conditions.

3.4. Capillary zone electrophoresis of H1 subtypes

Parameters of capillary zone electrophoresis were optimized for the separation of H1 histones (Kratzmeier et al., submitted). The retention times and the relative positions of the peaks of the different H1 subtype proteins in this system were determined by mixing experiments. Individual recombinant H1 subtype proteins were added to a total histone H1 preparation from HeLa cells and the positions of the peaks were determined by comparing the elution profiles with and without addition of recombinant H1 subtype proteins (Fig. 4). H1° eluted first and the other H1 histones eluted later but with only slightly different elution times. Histone H1.5 eluted first of the bulk of main type H1 histones with nearly the same retention time as H1.4. Since H1.5 generally is expressed at a lower level than H1.4, H1.5 often represents only a shoulder of the H1.4 peak. The retention time of H1.2 and H1.3 is also slightly different, therefore H1.3 and H1.2 form two closely associated peaks, but are clearly distinguishable. H1.1 elutes with the greatest retention time of the main type H1 histones as a single peak. The retention time of the testis specific H1t is much higher than that of the main type variants. The peaks of the elution profiles in Fig. 4 not related to any H1 histone subtype may represent other very basic acid soluble proteins of HeLa cells and/or modified H1 subtypes. The peak between H1.4 and H1.3, indicated in Fig. 4 by an asterisk, represents the high mobility group protein HMG17. On the basis of these mixing experiments, we determined that HeLa cells contain the H1 subtypes H1.4, H1.2, and low levels of H1.5.

4. Discussion

We expressed seven human H1 histone subtype proteins in relatively high levels in the yeast *Saccharomyces cerevisiae*. The isolation procedure was optimized for pure and high yield preparations. Due to the absence of endogenous standard H1 in yeast, this system allows the preparation of individual H1 subtype histones with no changes in their primary structures, i.e. without sequence tags. These proteins were used to calibrate two different systems for detecting H1 subtypes, 2D gel electrophoresis and capillary zone electrophoresis.

In contrast to the expression of sea urchin H1 histone in yeast ([33,34]; our unpublished data), we do not observe any differences on the growth behavior of the transformed yeast cells compared with mock transformed cells (only vector) even when the transformed cells expressed high levels of human H1 histones (data not shown).

In the case of H1° we localized the recombinant H1° protein in yeast by indirect immunofluorescence as speckles within the cytoplasm of the transformed cells and not in the nucleus (manuscript in preparation). This cytoplasmic localization may be the reason for the unchanged growth behavior. There were also no differences in growth between transformants expressing different subtypes or expressing the H1 histone genes at different levels. The human H1 histone genes were expressed as full length products (Fig. 2). We did not detect any proteolytic degradation products as described for the expression of *Xenopus laevis* linker histone H5 in yeast [22]. Expression of this linker histone gene in *Saccharomyces cerevisiae* resulted only in a shortened degradation product, whereas the mRNA was full length. As we found for the expression of the human H1 histones, the expression of this truncated linker histone in our system had also no effect on the growth behavior of the transformed cells (data not shown).

Recently Wellman et al. [35] described the expression of three mouse H1 histone genes (H1°, H1t and H1-1) using the prokaryotic *E. coli* expression system. The expressed mouse linker histones were partly degraded in *E. coli* even when protease inhibitors were added to the lysis buffer. The same problem has been reported by Gerchman et al. [21] who tried to express chicken linker histone H5 in the *E. coli* system. Bharath et al. [36] expressed the rat histone H1d (H1.3) in *E. coli* using a His-tag for purification on Ni-NTA-agarose. A subsequent chromatographic purification step on heparin agarose yielded a relatively pure recombinant linker histone in high quantity, but this protein contained the additional histidine-tag residues that may change its properties. Furthermore, expression of heterologous proteins in *E. coli* can result in a mixture of recombinant proteins differentially processed at their N-termini. Bacterial expression of an artificial gene encoding a repeated tripeptide aspartyl-phenylalanyl-lysine [37] resulted in a mixture containing proteins that retained the N-terminal formyl-methionyl or methionyl residue, whereas just a small portion was properly processed.

In our system, the expression rates of the individual human H1 subtype proteins varied within an order of magnitude. Expression of histone H1° resulted in a yield of 0.5 mg/l culture, whereas the other H1 histones including H1t gave lower yields with gradual differences (data not shown). Similar results were described for the expression of mouse linker histones in *E. coli*, where the expression of H1° histone was higher than that of H1t and H1-1 (H1.2) [35]. The high rate of H1° histone synthesis may be due to its higher lysine content compared with the main type linker histones, whereas the much lower expression rate of H1t histones may be due to the high arginine content of these subtypes. A similar explanation for the low bacterial expression rate of a linker histone that is rich in arginine has been discussed by Gerchman et al. [21] using the *E. coli* system for the expression of chicken H5.

The isolation protocol was optimized for rapid and high yield preparation of the linker histones. Two different methods were combined to disintegrate about all of the yeast cells of a preparation. Zymolyase treatment removed the cell wall of the cells in the logarithmic growth phase and altered the cell wall of the cells in the stationary phase with the effect that they were more readily broken by blending with glass beads. Blending with glass beads is necessary for the disruption of the integrity of the cell nuclei. In addition, direct lysis of the cells in 5% PCA avoids proteolytic degradation through inactivation of endogenous proteases by acidic denaturation. The combination of these two methods allowed us to grow the induced culture to a high cell density (OD_{600nm}: 4.0) and resulted in about 10 g cell mass from 1 l culture medium. The 5% PCA soluble proteins (mainly H1 histones) were collected by precipitation with 20% TCA. The alternative method of precipitating the linker histones with ice-cold acetone reduced

the background of contaminating proteins, but also the yield of recombinant linker histones.

The described expression and purification system allowed us to isolate pure H1 subtype proteins (Fig. 2) without expensive and time consuming chromatographic purification steps. The isolated subtype protein collection allowed us to calibrate the spots in the two-dimensional electrophoresis (Fig. 3) and the peaks of the capillary zone electrophoresis (Fig. 4). We were able to correlate an electrophoretically separated spot or peak, respectively, to the corresponding gene transcript, meaning the matching of transcriptional data with protein data without sequencing the proteins.

Until now several transcriptional data of the H1 histone genes on the one side and H1 histone protein patterns of cells on the other side have been reported, but correlation of the gene expression and the protein data was difficult [13]. On the other hand, we could show that the histone H1 subtype pattern of HeLa cells correlates well with the mRNA pattern in this cell line, which we had determined by RNase protection assay [38].

In summary, we have established a simple and fast expression and purification system for H1 linker histones that are not altered in their primary structure. The purified subtypes allowed us to calibrate a fast and simple two-dimensional electrophoresis system and to calibrate the elution profile of the capillary zone electrophoresis, an efficient separation system for low amounts of H1 histones. In addition to this application as an analytical tool, the expression of individual H1 subtype proteins in yeast provides the substrates for elucidating H1 histone subtype specific effects such as differential binding to specific DNA sequences, effects on transcriptional regulation and protein-protein interactions.

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