MOLECULAR AND CELLULAR BIOLOGY, Apr. 1994, p. 2822–2835
0270-7306/94/$04.00+0

Vol. 14, No. 4

Histone H1 Expressed in *Saccharomyces cerevisiae* Binds to Chromatin and Affects Survival, Growth, Transcription, and Plasmid Stability but Does Not Change Nucleosomal Spacing

CAROLINE LINDER AND FRITZ THOMA*

Institut für Zellbiologie, ETH-Hönggerberg, CH-8093 Zürich, Switzerland

Received 13 September 1993/Returned for modification 28 October 1993/Accepted 19 January 1994

Histone H1 is proposed to serve a structural role in nucleosomes and chromatin fibers, to affect the spacing of nucleosomes, and to act as a general repressor of transcription. To test these hypotheses, a gene coding for a sea urchin histone H1 was expressed from the inducible *GAL1* promoter in *Saccharomyces cerevisiae* by use of a YEp vector for high expression levels (strain YCL7) and a centromere vector for low expression levels (strain YCL1). The H1 protein was identified by its inducibility in galactose, its apparent molecular weight, and its solubility in 5% perchloric acid. When YCL7 was shifted from glucose to galactose for more than 40 h to achieve maximal levels of H1, H1 could be copurified in approximately stoichiometric amounts with core histones of Nonidet P-40-washed nuclei and with soluble chromatin fractionated on sucrose gradients. While *S. cerevisiae* tolerated the expression of low levels of H1 in YCL1 without an obvious phenotype, the expression of high levels of H1 correlated with greatly reduced survival, inhibition of growth, and increased plasmid loss but no obvious change in the nucleosomal repeat length. After an initial induction, RNA levels for GAL1 and H1 were drastically reduced, suggesting that H1 acts by the repression of galactose-induced genes. Similar effects, but to a lower extent, were observed when the C-terminal tail of H1 was expressed.

Most of the eukaryotic genome is packaged by histone proteins into nucleosomes, 30-nm chromatin fibers, and higher-order structures. The H1 class of histones includes a variety of subtypes or variants of lysine-rich proteins with a short N-terminal tail, a central globular domain, and a long, highly charged C-terminal tail. H1 is proposed to serve different roles (for a review, see reference 62). (i) At the nucleosome level, DNA is wrapped in two superhelical turns around an octamer of core histones (two each of H2A, H2B, H3, and H4). Our current view is that on the average, one histone H1 molecule binds from the outside at the entry and exit site of the linker DNA and stabilizes two turns of DNA in the nucleosome. This view is supported by electron microscopy (16, 57), protein-protein cross-linking (8) and, in particular, by the observation of a transient stop at about 160 bp during chromatin digestion with micrococcal nuclease (38) and the subsequent isolation of the corresponding particle, the chromatosome (47). More recent reports indicated 168 bp for the chromatosome stop (1). However, there are additional binding sites for H1 (39, 53) since, for example, chicken erythrocyte chromatin was shown to accommodate 1.3 H1 proteins per nucleosome (H1 plus H5), indicating that some nucleosomes must have two molecules of H1 proteins (5). The fraction of nucleosomes that bind H1 at the entry and exit site is unknown and might vary in different organisms or tissues (53). (ii) Histone H1 might affect the spacing of nucleosomes. This role was suggested by in vitro reconstitution experiments (25, 61) and by the observation that changes in histones, in particular linker histone H1, during the development of the sea urchin correlated with a change in the nucleosomal repeat length (2). (iii) Histone H1 stabilizes and organizes the folding of nucleosomes into 30-nm chromatin fibers (57). (iv) Histone H1 is regarded as a repressor of transcription both in vivo and in vitro. As a prominent example, partial replacement of histone H1 by histone H5, a variant of H1, during the maturation of avian erythrocytes might repress transcriptional activity, possibly by the formation of more stable nucleosomes and higher-order structures (4, 37, 58). The structural and functional roles of the individual domains of H1 are less clear. The globular region appears to be sufficient for sealing the nucleosome in vitro (1) and was suggested to organize the 30-nm fibers (33, 58). The N-terminal tail and, in particular, the positively charged C-terminal tail might bind to linker DNA or adjacent nucleosomes and thereby contribute to the condensation of chromatin fibers (27, 58). This description of the roles of H1 is based largely on in vitro experiments.

A direct approach to testing the roles of histone H1 involves the expression of H1 genes in living cells. The expression of an inducible transfected H5 gene in rat sarcoma cells resulted in the arrest of cell proliferation and changes in gene expression but not in an altered nucleosomal repeat length (50, 51). In these experiments, however, the additional H5 protein had to compete with the endogenous H1 proteins. To test the possible roles of histone H1 in the absence of other H1 proteins, we expressed a sea urchin H1 in the yeast *Saccharomyces cerevisiae*. *S. cerevisiae* has no endogenous histone H1. While the composition and structure of the yeast nucleosome core are very similar to those of higher eukaryotes (6), the nucleosomal repeat is only about 160 bp long (7, 30, 59) and therefore is too small to represent an octamer of core histones plus histone H1. However, mapping of nucleosome positions showed that individual nucleosomes can be tightly packed while others can be well spaced and accommodate more than 170 bp (52, 54). We showed that a sea urchin H1 could be expressed at low and high levels (in amounts similar to those of core histones) and that it copurified with nuclei and bound to chromatin. While low levels of H1 were tolerated, high levels affected survival,

* Corresponding author. Phone: 01-633-3323 or 01-633-3352. Fax: 01-371-2894.

growth, transcription, and plasmid stability, but the nucleosomal repeat was not altered.

MATERIALS AND METHODS

Enzymes and chemicals. Restriction enzymes, micrococcal nuclease, leupeptin, and pepstatin were from Boehringer. Phenylmethylsulfonyl fluoride (PMSF) was from Merck, perchloric acid (PCA, 70%) and Ficoll 400 were from Fluka AG, Nonidet P-40 (NP-40) was from Sigma, Zymolyase 100T was from Seikagaku Kogyo Co. Ltd., and [5,6-^{3}H]uracil was from Amersham.

Plasmids. The H1 gene was isolated as an *Hpa*I-*Hin*dIII fragment (1,130 bp) from the cloned early histone gene cluster h22 of the sea urchin *Psammechinus miliaris* (43) (a gift from M. L. Birnstiel and M. Busslinger, Institute of Molecular Pathology, Vienna, Austria). pCL1 is a pUC8 derivative which contains the *GAL1-GAL10* promoter as an *Eco*RI-*Bam*HI fragment (678 bp) from pBM150 (23) inserted between the *Eco*RI and *Bam*HI sites and the H1 gene (*Hpa*I-*Hin*dIII fragment) inserted between the *Hin*cII and *Hin*dIII sites. The *Eco*RI-*Hin*dIII fragment of pCL1 containing the *GAL1-GAL10* promoter and the H1 gene was used to replace the *Eco*RI-*Hin*dIII fragment of pBM272 (containing the *GAL1-GAL10* promoter) to yield YCpCL2 (Fig. 1). (pBM272 is a derivative of pBM150 containing an additional *Hin*dIII site and was kindly provided by M. Johnston.) The same *Eco*RI-*Hin*dIII fragment of pCL1 was inserted between the *Hin*dIII and *Eco*RI sites of YEp352 (20) to yield the 2μm yeast plasmid YEpCL11. YEpCL12 is YEpCL11 from which the *Bam*HI-*Pst*I fragment was removed to delete the H1 gene. YEpCL13 is YEpCL11 from which the *Bam*HI fragment was deleted to result in expression of the C-terminal tail (from amino acids 89 to 206; see Fig. 10).

Yeast strains and media. The yeast strains used in this study are listed in Table 1. YM262 (*MATα ura3-52 his3-200 ade2-101 lys2-801 tyr1-501*) was used as a host strain (23) (kindly provided by M. Johnston). YM262 was transformed by the lithium acetate method (21), and clones were selected on minimal media containing glucose. Yeast media were made as described previously (45). The complex medium (YPD) contained 1% Bacto Yeast Extract, 2% Bacto Peptone, 2% glucose, and 0.003% adenine sulfate. The synthetic minimal medium contained 0.67% Bacto Yeast Nitrogen Base without amino acids but supplemented with adenine at 20 mg/liter, L-histidine at 20 mg/liter, L-lysine HCl at 30 mg/liter, and L-tyrosine at 30 mg/liter. As a carbon source, 2% glucose (SD), 2% galactose (SG), or a mixture consisting of 3% glycerol with 2% lactate (glycerol-lactate) or of 3% glycerol with 2% lactate and 2% galactose (glycerol-lactate-galactose) was added. For plates, 2% Bacto Agar was added before autoclaving.

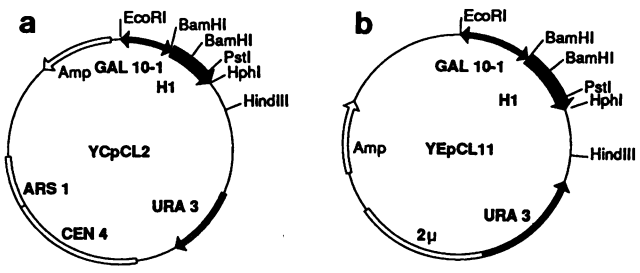

FIG. 1. Constructs. (a) YCpCL2 contains a sea urchin histone H1 gene linked to the *GAL1* promoter of host vector pBM272. The plasmid occurs at low copy numbers and allows the expression of H1 at low levels in galactose-containing media. (b) YEpCL11 contains a sea urchin histone H1 gene linked to the *GAL1* promoter of vector YEp352. The plasmid occurs at high copy numbers and allows high levels of H1 expression in galactose-containing media. ARS 1 and 2μ indicate origins of replication; URA 3 and Amp are the marker genes for selection in *S. cerevisiae* and in *Escherichia coli*, respectively. Relevant restriction sites are indicated.

TABLE 1. Strains and plasmids used in this study[a]

Yeast strain	Plasmid	Property
YCL1	YCpCL2	Expression of H1 at low levels
YCL7	YEpCL11	Expression of H1 at high levels
YM262/pBM272	pBM272	Control strain with host plasmid
YM262/YEp352	YEp352	Control strain with host plasmid
YCL21	YEpCL12	Control strain with *GAL1-GAL10* promoter plasmid
YCL22	YEpCL13	Expression of C-terminal tail of H1 at high levels

[a] The host strain was YM262 (*MATα ura3-52 his3-200 ade2-101 lys2-801* tyr1-501) (23).

Galactose induction. For shifting cells from glucose to galactose, yeast cultures were grown in SD medium (30°C), harvested by centrifugation, washed in water (at room temperature), resuspended in the same volume of prewarmed SG medium, and further incubated at 30°C. For galactose induction in glycerol-lactate, galactose was added directly to yeast cultures grown in glycerol-lactate.

Cell survival and mitotic plasmid stability assays. The cell density was determined by counting cells in a hemocytometer. A given number of cells was plated in triplicate on YPD and SD plates (100 to 200 cells per plate) and incubated at 30°C. Survival was calculated as the number of colonies counted on YPD plates as a percentage of the number of cells plated (100%). Mitotic plasmid stability was calculated as the number of colonies growing on selective SD plates as a percentage of the number of colonies growing on YPD plates (100%). Cells that lose the expression plasmid and hence the selection marker *URA3* during mitotic growth cannot grow on selective plates but can grow on YPD plates.

Preparation of crude NP-40-washed nuclei (NP-40 nuclei). Nuclei were prepared by the protocol of Davie et al. (13) with modifications (37a). One-liter cultures of yeast cells were grown in SD medium to 1.6×10^7 to 2.0×10^7 cells per ml or grown in SD medium to 1.6×10^7 to 2.0×10^7 cells per ml and transferred into the same volume of SG medium. The cells were harvested by centrifugation in a GSA3 rotor (Sorvall, Du Pont Instruments) (5 min, 4,000 rpm, room temperature), washed twice in H_2O by resuspension and centrifugation, and collected in a 50-ml Falcon tube to yield about 1.5 to 1.8 g (wet weight) and a volume of 1 to 1.5 ml (1 volume). The cells were resuspended in 3 volumes of prewarmed (30°C) 20 mM EDTA (pH 8)–7 mM β-mercaptoethanol, incubated with mild agitation (60 rpm) at 30°C for 15 min, collected by centrifugation in a Sorvall RC5-B centrifuge (Du Pont Instruments) (3 min, 3,000 rpm, room temperature), washed in 2 volumes of prewarmed (30°C) 1 M sorbitol, collected by centrifugation, and resuspended in 3 volumes of prewarmed (30°C) spheroplasting solution (1 M sorbitol, 5 mM β-mercaptoethanol). Zymolyase 100T (4 mg) was added, and the cell suspension was agitated (60 rpm) at 30°C for 10 to 20 min (for cultures grown in SD medium) or for 20 to 30 min (for cultures grown in SG medium). Spheroplasting was monitored by microscopy and considered to be complete when 90 to 95% of one aliquot of the cells could be lysed by the addition of 1% sodium dodecyl

sulfate (SDS) (visible as a transparent "ghost"). The following steps were done on ice, and all materials and solutions were precooled to 4°C. Spheroplasts were harvested by centrifugation (3 min, 3,000 rpm), washed with 3 volumes of 1 M sorbitol, recollected, and lysed in 16 ml of lysis buffer [18% Ficoll (wt/vol), 20 mM KPO_4, 1 mM $MgCl_2$, 0.25 mM EDTA, 0.25 mM ethylene glycol-bis(β-aminoethylether)-*N,N,N',N'*-tetraacetic acid (EGTA), 1 mM PMSF, 1 μg of pepstatin per ml, 1 μg of leupeptin per ml; protease inhibitors were added immediately before use] by vortexing and shaking twice each for 1 min and then by four strokes in a 60-ml Dounce homogenizer. Lysed spheroplasts were centrifuged in an SS-34 rotor (Sorvall, Du Pont Instruments) (5 min, 5,700 rpm), the turbid supernatant containing crude nuclei was transferred to an SS-34 tube by decantation (the loosely packed upper part of the pellet was partly transferred), and the nuclei were pelleted in a Sorvall RC5-B centrifuge (20 min, 16,000 rpm). The crude nuclear pellets were homogeneously resuspended by pipetting into 1.5 ml of NP-40 solution (10 mM Tris-HCl [pH 8], 75 mM NaCl, 0.5% NP-40, 1 mM PMSF, 1 μg of pepstatin per ml, 1 μg of leupeptin per ml; NP-40 and protease inhibitors were added immediately before use) and incubated for 30 min. Spectra run in 1% SDS (100 times the dilution of the suspension) showed a clear peak at 260 nm (the A_{260} was ~50). The nuclei were collected by centrifugation in an SS-34 rotor (10 min, 16,000 rpm), and the supernatants were discarded. The NP-40 wash was repeated twice, but the pellets were only partially resuspended, incubated for 15 min, and collected by centrifugation at 10,000 rpm (10 min). The pellet containing crude NP-40 nuclei was resuspended in 1.0 ml of TE (10 mM Tris, 1 mM EDTA [pH 8]) and immediately used for further experiments.

Histone H1 extraction from crude NP-40 nuclei with 5% PCA. PCA extraction was done as described previously (41). All steps were done on ice, and materials and solutions were precooled to 4°C. Crude NP-40 nuclei were transferred to a 13-ml Falcon tube. One volume of 10% PCA (prepared immediately before use) was added. The suspension was vortexed for 10 s, incubated for 30 min, and pelleted by centrifugation in an HB-4 rotor (Sorvall, Du Pont Instruments) (20 min, 10,000 rpm), and the supernatant containing histone H1 protein was transferred to a 15-ml Corex tube. The pellet was resuspended in 5% PCA, incubated for 15 min, and repelleted (15 min, 10,000 rpm). The second supernatant was pooled with the first supernatant. To precipitate the soluble histone H1 protein, 3.5 volumes of acetone (−20°C) and HCl (37%) were added (28.5 μl/ml of supernatant). The suspension was mixed by vortexing and incubated at −20°C overnight. The precipitate was collected by centrifugation in an HB-4 rotor (30 min, 10,000 rpm). The pellet was washed with acetone (−20°C) and dried under vacuum. The H1 precipitate was resuspended in 1× SDS sample buffer (see below) for protein gel electrophoresis.

Protein gel electrophoresis. Proteins were analyzed on SDS–15% polyacrylamide gels as described previously (26) but with modifications (57). Samples were dissolved in SDS sample buffer (0.0625 M Tris-HCl [pH 6.8], 2% SDS, 10% glycerol, 2.5% β-mercaptoethanol, traces of bromphenol blue) and heat denatured. Silver staining of the gels was done as described previously (64).

Extraction of soluble chromatin. The following steps were done on ice, and materials and solutions were precooled to 4°C. NP-40 nuclei were prepared as described above but resuspended in buffer A (20 mM Tris-HCl [pH 8], 150 mM NaCl, 5 mM KCl, 1 mM EDTA, 1 mM PMSF [54]) to yield an A_{260} of 0.6 to 0.7. The nuclei were adjusted to 5 mM $CaCl_2$ and incubated with 180 U of micrococcal nuclease for 2.5 or 1 min at 37°C. The digestion was terminated by the addition of EDTA (pH 8) to a final concentration of 8 mM, and the suspension was centrifuged in an HB-4 rotor (10 min, 10,000 rpm) to yield a pellet, designated pellet 1, and a supernatant, designated supernatant 1, containing soluble chromatin. Further incubation of pellet 1 in 10 mM Tris (pH 8)–1 mM EDTA–10 mM NaCl for 12 h did not release more soluble chromatin. Supernatant 1 (1.8 ml) was fractionated on a linear 5 to 15% (wt/vol) sucrose gradient (in 10 mM Tris [pH 8]–1 mM EDTA–10 mM NaCl) with an SW-41 rotor (Beckman) at 20,000 rpm for 14 h (4°C) in a Beckman L70 ultracentrifuge. Fractions of 1.2 ml were collected. Half of the fractions were used for either protein or DNA analysis. The proteins were precipitated with 25% trichloroacetic acid (TCA) for 30 min and collected with a microcentrifuge. The pellet was washed with 200 μl of acetone (−20°C), recollected by centrifugation, vacuum dried, and finally resuspended in 25 μl of SDS sample buffer. For DNA analysis, chromatin was precipitated by the addition of 66 μl of 3 M sodium acetate and 2.5 volumes of ethyl alcohol (−20°C), incubation overnight at −20°C, and centrifugation in a microcentrifuge (30 min). The pellet was washed with 200 μl of 80% ethyl alcohol, recollected, and vacuum dried. The pellet was resuspended in 20 μl of 10 mM Tris–1 mM EDTA and adjusted with TBE loading buffer (89 mM Tris, 89 mM boric acid, 20 mM EDTA, 8% glycerol, 0.1% bromphenol blue, 0.1% xylene cyanol) containing 1% SDS. The samples were incubated at 37°C for 10 min before being loaded on a 1.5% agarose gel.

Chromatin analysis with micrococcal nuclease. Preparation of genomic chromatin, digestion with micrococcal nuclease, DNA extractions, and agarose gel electrophoresis were carried out as described previously (7), with the exception that 0, 7, 15, 22, 37, 45, 52, and 105 U of micrococcal nuclease per ml was used.

Determination of RNA synthesis rates. The rates of RNA synthesis were determined by pulse labelling the RNA with [^{3}H]uracil as described previously (10). Aliquots of 2×10^7 cells were taken 0, 16, 20, 24, 28, 40, and 45 h after shifting of the cells from SD to SG medium or 0, 3, 6, and 9 h after galactose induction in glycerol-lactate. The cells were incubated in the presence of 5 μCi of [^{3}H]uracil for 10 min at 30°C, and incorporated radioactivity was counted after precipitation with TCA.

RNA analysis. Cells were grown and harvested as described below (see the legend to Fig. 8). Total RNA was extracted (22), run on 1% agarose gels containing formaldehyde (60), blotted on Biodyne A membranes according to manufacturer instructions (Pall), and hybridized to the appropriate probes. The membranes were exposed on Fuji RX films with one intensifying screen. The following DNAs were used to generate probes: a *Cla*I fragment of pYA301 for *ACT1* (kindly provided by D. Gallwitz), an *Eco*RI fragment of pNN77 for *GAL1* (kindly provided by R. Davis), an *Ava*II-*Sau*3A fragment of the *URA3* gene, and a pBR322 derivative containing sequences that hybridize to Ty elements (kindly provided by P. Philippsen). DNA was radioactively labelled by use of a random priming kit (Pharmacia).

RESULTS

To investigate the roles of histone H1 in *S. cerevisiae*, we attempted to express sufficient histone H1 proteins to yield approximately one H1 molecule per nucleosome. For this study, the H1 gene isolated from the cloned early histone gene cluster (h22) of *P. miliaris* (43) was used. For brevity, this gene is called the h22-H1 gene. Since the expression of H1 was

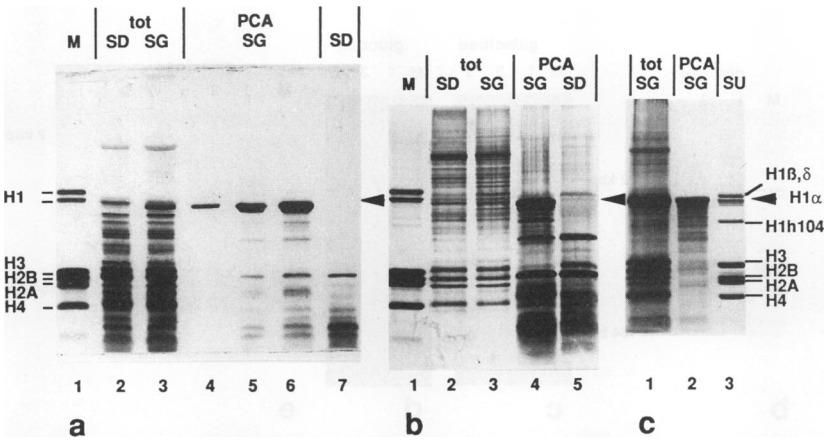

FIG. 2. Expressed H1 is copurified with NP-40 nuclei. NP-40 nuclei were prepared from YCL7 cells (a and c) and YCL1 cells (b) grown in selective media containing glucose (SD) or galactose (SG). YCL7 was incubated in SG for 40 h. YCL1 was grown on to an A_{600} of 0.8. Total proteins (tot) and 5% PCA-extracted proteins (PCA) were visualized by SDS-polyacrylamide gel electrophoresis and silver staining. A comparison of YCL7 proteins with sea urchin histones (SU) is shown in panel c. Rat liver histones (H1, H3, H2B, H2A, and H4) were used as markers (M). H1α is the product of the sea urchin h22-H1 gene (7a). The arrowhead marks the position of expressed H1. Lanes contained the following approximate amounts of DNA, in micrograms (assuming 1.6 μg of DNA per 10^8 haploid yeast cells and no loss during nucleus preparation): (a) 2 (lanes 2 and 3), 3 (lane 4), 14 (lane 5), and 28 (lanes 6 and 7); (b) 0.4 (lanes 2 and 3) and 200 (lanes 4 and 5); (c) 2 (lane 1) and 20 of an old sample (lane 2). Rat liver histones were used at 0.75 μg (a, lane 1) and 0.5 μg (b, lane 1). Two micrograms of sea urchin histones was used (c, lane 3).

expected to be deleterious for the cells, the sea urchin histone H1 gene (h22) was fused to the inducible *GAL1* promoter. This promoter allows the cells to be cloned and grown in glucose, in which transcription is repressed, and allows the expression of H1 after shifting of the cells to galactose-containing medium. Two constructs and the corresponding yeast strains and control strains are shown in Fig. 1 and Table 1. YCpCL2 is based on the centromeric expression vector pBM272, which occurs in low copy numbers. YEpCL11 is based on the 2μm vector YEp352 which, in the presence of 2μm circles, occurs at high copy numbers. YCpCL2 and YEpCL11 were used to transform *S. cerevisiae* YM262 to yield strains YCL1 and YCL7, respectively. The copy numbers determined by Southern blotting were about 1 to 2 for YCpCL2 and approximately 50 for YEpCL11 (data not shown). YM262/pBM272 and YM262/YEp352 are the appropriate control strains containing the host vectors. YCL21 is an additional control strain used to test for possible effects of transcription factor titration and transcriptional interference during growth in galactose. It contains YEpCL12, a derivative of YEpCL11 from which the H1 gene has been deleted (*Bam*HI-*Pst*I).

H1 is expressed, is not degraded, and copurifies with nuclei. To investigate whether the H1 protein was expressed and transported into nuclei, crude nuclear pellets were prepared from spheroplasts lysed in Ficoll. To visualize the histone proteins in the nuclei by gel electrophoresis, loosely associated proteins (ribosomes and putative cytoplasmic contaminants) were removed by three washes of the nuclei with NP-40 (29) (NP-40 nuclei). The total protein pattern of NP-40 nuclei extracted from YCL1 and YCL7 cells grown in glucose or shifted to galactose showed the expected low-molecular-weight bands characteristic of the core histones (H2A, H2B, H3, and H4; Fig. 2; see also Fig. 6) (29, 59). When YCL7 cells were shifted for 40 h to galactose to produce maximal amounts of H1 (for detailed kinetics, see below), the protein pattern of NP-40 nuclei revealed one additional, strong band (Fig. 2a, compare SG and SD). This additional band was identified as the expressed sea urchin H1 histone on the basis of the following criteria. (i) This band was visible in YCL7 cells grown in galactose, in which the *GAL1* promoter is active, and not in glucose. (ii) As characteristic for an H1 protein, the induced h22-H1 gene product was soluble in 5% PCA (Fig. 2a, lanes 4 to 6). (iii) The apparent molecular weight of the H1 band was indistinguishable from that of the H1α band of sea urchin histones (Fig. 2c). H1α is the product of the h22-H1 gene in the sea urchin. The induced protein was shorter than rat liver histone H1, consistent with a shorter open reading frame of 206 amino acids in the h22-H1 gene (7a). Hence, the sea urchin H1 histone expressed in *S. cerevisiae* remains intact and is not degraded. Some degradation was observed only in old cultures (see below).

On the basis of the silver staining of the protein gel, the amount of histone H1 that was copurified with NP-40 nuclei from YCL7 cells was similar to the amounts of the individual core histones. Core histones and H1 stained similarly, as shown by the marker lanes containing sea urchin and rat liver histones. This result suggests that large amounts of sea urchin H1 were expressed in YCL7 cells and transported into nuclei. In contrast, significantly less protein was produced when we used a centromere vector in YCL1 cells grown in galactose. In NP-40 nuclei of YCL1, no H1 band was detectable by silver staining of the protein gel (Fig. 2b). PCA extraction of NP-40 nuclei and a roughly 100-fold concentration were required in order to detect the expression of H1. Gene dosage-dependent expression levels—high in YCL7 and low in YCL1—can be considered a fourth criterion for identifying histone H1.

Expressed H1 binds to chromatin. To investigate whether expressed H1 enters the nuclei and binds to chromatin, the NP-40 nuclei of galactose-induced YCL7 cells were digested with micrococcal nuclease for 2.5 min and for 1 min to yield short and long soluble chromatin fragments, respectively. The insoluble material was pelleted by centrifugation; the supernatant contained the released soluble chromatin fragments. Further incubation of the pellet in low-ionic-strength buffer (10 mM Tris, 1 mM EDTA) did not solubilize more chromatin

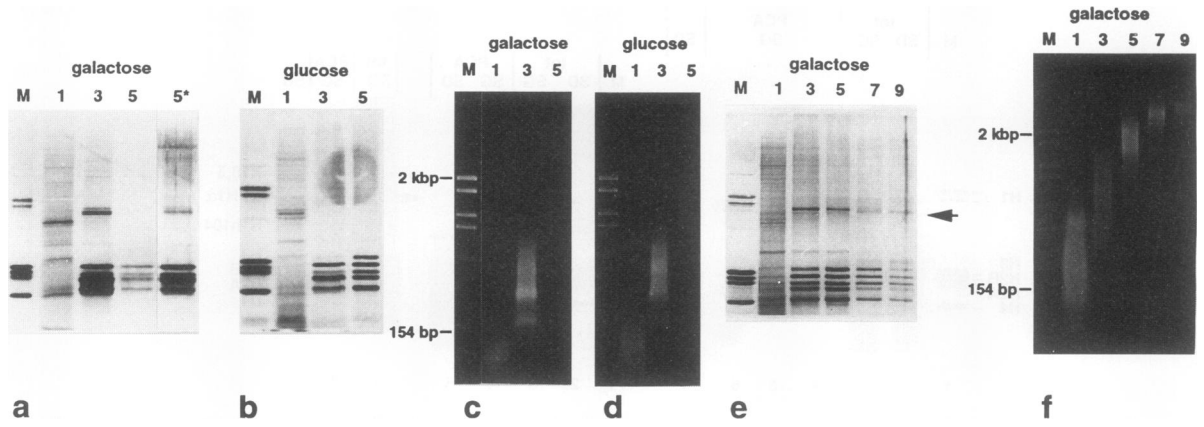

FIG. 3. H1 is bound to soluble chromatin. YCL7 cells were grown in galactose for 41 h (a, c, e, and f) or in glucose (b and d), and NP-40 nuclei were digested with micrococcal nuclease for 2.5 min (a, b, c, and d) or 1 min (e and f) to yield short and long chromatin fragments, respectively. Chromatin was fractionated on a 5 to 15% glucose gradient. The protein composition and DNA content of individual fractions were visualized by SDS-polyacrylamide gel electrophoresis and silver staining (a, b, and e) and agarose gel electrophoresis and ethidium bromide staining (c, d, and f), respectively. Numbers (1 to 9) indicate gradient fractions from top to bottom. The arrow indicates H1. Rat liver histones were used as markers for protein gels (M). pBR328 DNA digested with *Bgl*I and pBR328 DNA digested with *Hin*fI were used as size markers for the DNA gels (M). Only a small amount of chromatin was present in fraction 5 of the galactose preparation. Lane 5* shows stronger staining of lane 5 (panel a). The optical density profiles of the gradients at 254 nm (data not shown) were dominated by RNAs and their digestion products in the top fractions, as indicated by RNA smears in fraction 1 (panels c, d, and f).

fragments. The chromatin fragments (in the supernatant) were fractionated according to size on a shallow sucrose gradient, and the fractions were analyzed for protein composition and DNA content (Fig. 3). Indeed, no soluble H1 was found on top of the gradient (Fig. 3a and e, lanes 1), but all fractions containing DNA also contained core histone proteins and H1 (Fig. 3a and e, lanes 3 to 9). No H1 was detected in soluble chromatin fractions of YCL7 cells grown in glucose (Fig. 3b). We conclude that the expressed H1 protein was bound to chromatin.

With respect to the H1-core histone stoichiometry, two points need to be discussed. (i) The H1/core histone ratio was somewhat lower in the fractions containing chromatin than in NP-40 nuclei. Since the amount of H1 expression is time dependent (see also Fig. 6), it is possible that a nonmaximal amount of H1 was produced at the time of harvest 41 h after induction. Alternatively, small fragments of chromatin may have been partially depleted of histone H1 during preparation (40). (ii) The H1 content of long chromatin appeared to be lower than the H1 content of short chromatin (compare fraction 5 [lane 5*] with fraction 3 [lane 3] in Fig. 3a or fraction 5 with fraction 3 in Fig. 3e). One reason might be inappropriate recovery and staining of fractions containing only very little material (e.g., fraction 5 in Fig. 3a). Alternatively, it should also be considered that longer chromatin fragments with a higher H1 content might not have been soluble under the conditions used and might have been precipitated in the pellet fraction.

Despite the low temperature and the presence of proteinase inhibitors during the preparation of nuclei, some degradation of core histones could not be avoided. For example, in fraction 5 of the glucose preparation, H3 was cleaved to yield an additional band between H4 and H2A, while in fraction 3, the cutting of H3 was almost complete, indicating that some proteinase activity accumulates toward the top of the gradient.

H1 expression affects growth, survival, and plasmid stability. To test for growth properties, YCL1 and YCL7 and control strains YM262/pBM272, YM262/YEp352, and YCL21 were spotted on selective plates containing glucose or galactose as the carbon source (Fig. 4). All strains grew on glucose within 3 days. On galactose, however, only YCL1 and the control strains grew within 4 days, while the growth of YCL7 was severely retarded. Only after 7 days of incubation were some YCL7 colonies visible (slowly growing colonies). There were too many colonies for the colonies to be considered revertants or mutants in the expression system. A similar retardation of YCL7 growth in the presence of galactose was observed when cells were spotted on selective plates containing either glycerol-lactate or glycerol-lactate plus galactose, except that longer

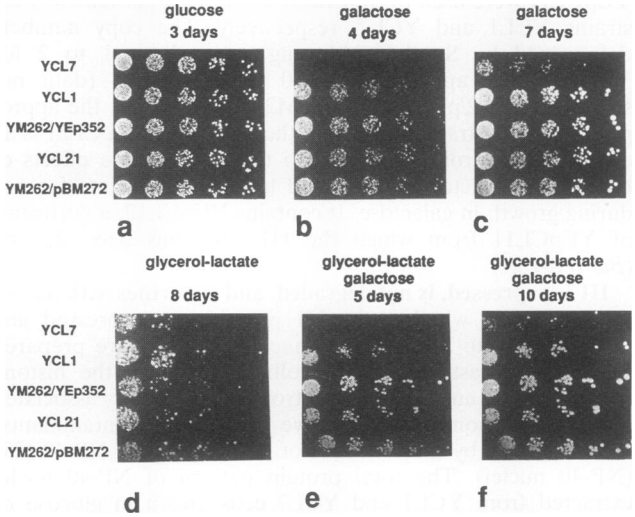

FIG. 4. H1 expression affects growth on plates. YCL7, YCL1, and control strains YM262/YEp352, YCL21, and YM262/pBM272 were grown in glucose or glycerol-lactate, and aliquots were spotted on synthetic media containing glucose, galactose, glycerol-lactate, and glycerol-lactate plus galactose. Incubation was done at 30°C. Approximate cell numbers spotted were, from left to right, 900, 180, 90, 18, and 9. The growth of YCL7 on galactose was severely retarded.

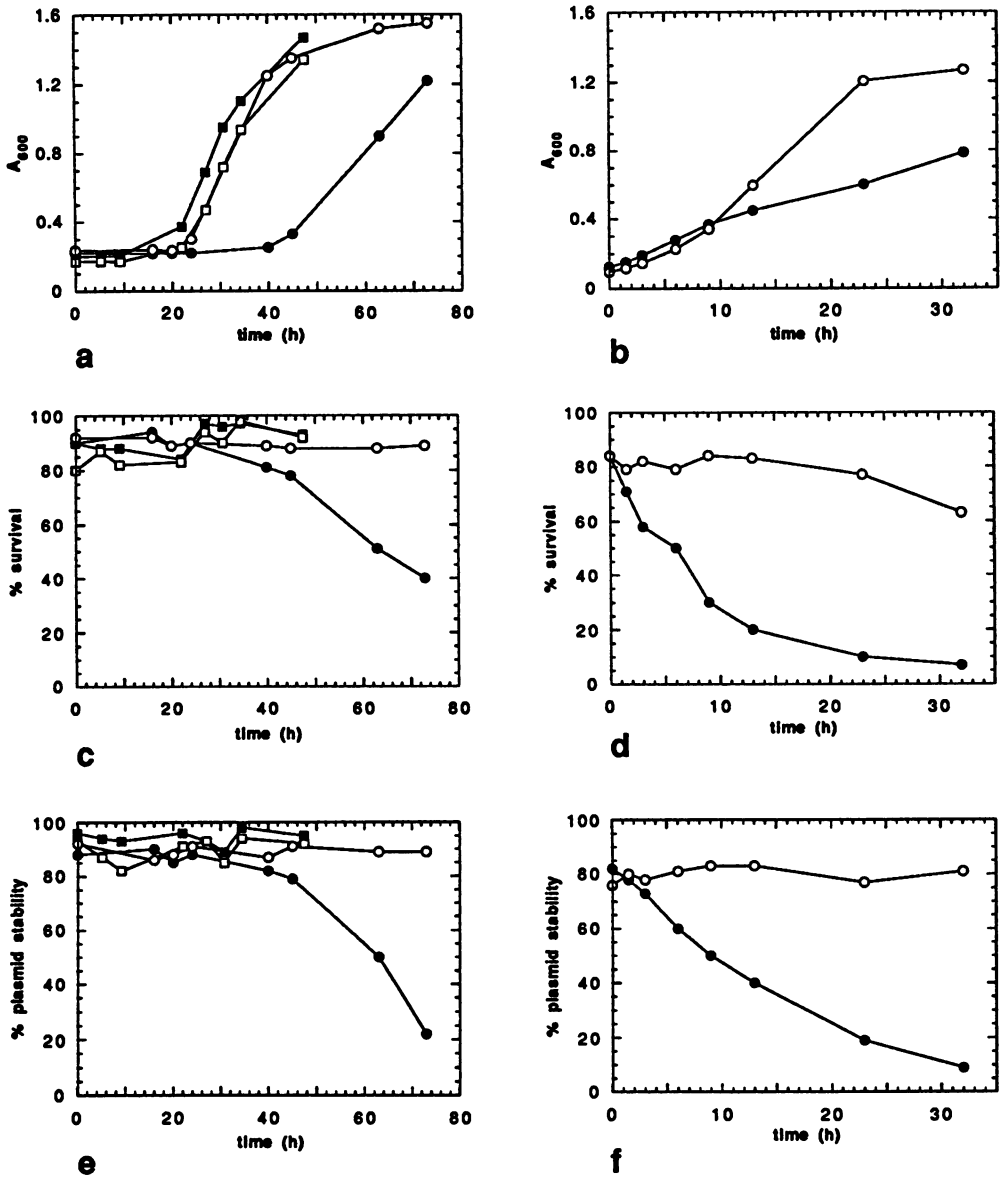

FIG. 5. H1 expression affects growth, survival, and plasmid stability. YCL1 (■), YCL7 (●), and control strains YM262/pBM272 (□) and YM262/YEp352 (○) were grown in selective media containing glucose to an A_{600} of 0.2, pelleted, and resuspended in selective media containing galactose (a, c, and e). Alternatively, galactose was added to YCL7 and YM262/YEp352 cells grown in glycerol-lactate to an A_{600} of 0.1 (b, d, and f). Growth was recorded as a function of time (A_{600}; a and b). Survival was monitored by spreading given numbers of cells (counted; 100%) on YPD plates and counting the colonies after 3 days (c and d). The mitotic stability of the plasmids was recorded by spreading equal amounts of cells on selective glucose plates (lacking uracil) and on YPD plates (e and f); the number of colonies on selective plates relative to the number of colonies on YPD plates (100%) is shown as the percentage of surviving cells containing the *URA3* marker gene and therefore the expression plasmid.

incubation times were required for the development of colonies. Inhibition of YCL7 growth was also apparent in liquid galactose-containing medium (Fig. 5a). While control strains and YCL1 resumed growing approximately 20 h after a shift from glucose to galactose, YCL7 started growing about 40 h after the shift. A reduced growth rate for YCL7 cultures was also observed when galactose was added to a glycerol-lactate-containing medium (Fig. 5b). It could be argued that high copy numbers of YEpCL11 might titrate transcription factors for galactose-regulated genes and thereby affect growth or that transcription from the *GAL1-GAL10* promoter might interfere with transcription of the marker gene *URA3*. This, however, is not the case, since control strain YCL21, which hosts the control plasmid YEpCL12 containing the *GAL1-GAL10* promoter but not the H1 gene, grew normally (Fig. 4; see also Fig. 10d). The results obtained on plates and in liquid media strongly suggested that high levels of H1 are deleterious (YCL7 cells), while low levels of H1 are tolerated (YCL1 cells).

A detailed time course of H1 expression for YCL7 cells is shown in Fig. 6. The proteins found in NP-40 nuclei at different times after galactose induction are shown. Little H1 was

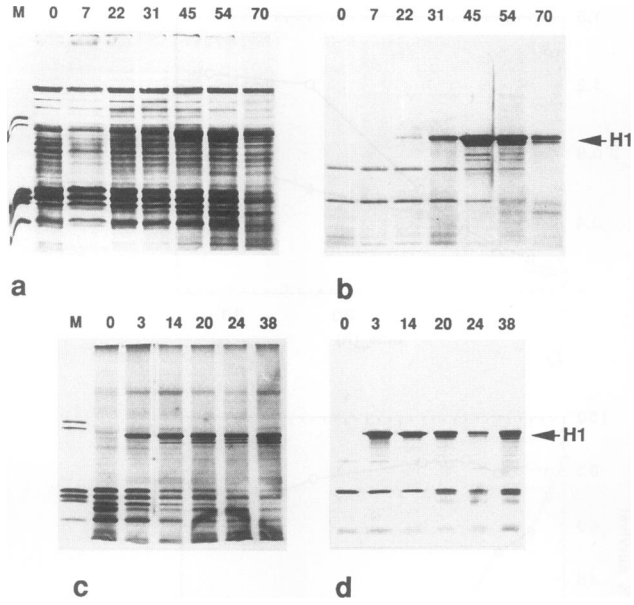

FIG. 6. Time course of H1 expression and accumulation in NP-40 nuclei. YCL7 cells were grown in SD to an A_{600} of 0.6 and shifted to SG (a and b). Alternatively, YCL7 was grown in glycerol-lactate to an A_{600} of 0.25, and galactose was added (c and d). NP-40 nuclei were prepared at several time points after induction (0 to 70 h) (indicated above lanes). Total proteins in NP-40 nuclei (a and c) and proteins soluble in 5% PCA (b and d) were visualized by SDS-polyacrylamide gel electrophoresis and silver staining. Panels contained the following approximate amounts of DNA, in micrograms (assuming no loss during nucleus preparation): (a) 1.6, (b) 42, (c) 1.3, and (d) 8. The position of expressed H1 is marked by an arrow. M, markers.

detected 22 h after a shift from glucose to galactose (Fig. 6b), at the time when control cells started to grow but the growth of YCL7 cells was inhibited (Fig. 5a). A maximal amount of H1 with respect to the amounts of core histones was observed approximately 45 h after the shift from glucose to galactose. This slow H1 production is consistent with the reduced H1 RNA levels (see below). After longer incubations, some degradation of core histones and of histone H1 was observed, indicating that at those times, some cells were dead. When YCL7 cultures were induced in glycerol-lactate, a similar amount of H1 with respect to the amounts of core histones was observed after 3 h of induction, but significantly more histone H1 than core histones was seen after 14 h, and degradation in particular of core histones occurred after about 20 h (Fig. 6c and d).

We tested the percentage of cells that recover after H1 expression and survive by plating given numbers of cells (counted; 100%) on a complex medium (YPD) and counting colonies that appeared (Fig. 5c and d). While control and YCL1 cells showed about 90% survival throughout the time course after a shift from glucose to galactose, the survival of YCL7 cells dramatically dropped after 40 to 45 h (Fig. 5c). After a shift from glycerol-lactate to glycerol-lactate plus galactose, the survival of control cells remained high (about 80%), while the survival of YCL7 cells dropped immediately, consistent with H1 expression without a lag phase and the reduced growth rate. These results show that H1 expression at high levels severely affects the survival of yeast cells, while low levels are tolerated.

Since H1 binds to chromatin and since an unbalanced overexpression of histones was shown to promote chromosome loss (35), the effect of H1 expression on plasmid stability was investigated (Fig. 5e and f). During the time course of H1 expression, one aliquot of cells was plated on nonselective plates containing glucose to yield colonies of surviving cells independent of the presence or absence of the expression plasmid (100%). Another aliquot was plated on selective plates containing glucose to yield colonies of cells that maintained the plasmid and recovered from H1 expression. While control and YCL1 cells maintained the expression plasmid throughout the time course of incubation in galactose (Fig. 5e and f), YCL7 cells showed a dramatic loss of the plasmids 40 to 45 h after a shift from glucose or immediately after a shift from glycerol-lactate. We conclude that H1 expression at high levels severely affects plasmid stability and maintenance.

H1 expression and transcription. Since H1 was postulated to serve as a general repressor of transcription, the incorporation of radioactive uracil into RNA was measured in 10-min pulses at different time points of incubation in galactose (Fig. 7). RNA synthesis was high in exponentially growing control cells and low when control cells entered stationary phase. In H1-expressing YCL7 cells, RNA synthesis was low after a shift of the culture from glucose to galactose and resumed higher rates at about 24 h, somewhat before the increase in cell numbers was observed. Similarly reduced RNA synthesis rates were observed when H1 synthesis in YCL7 cultures was induced in glycerol-lactate-containing medium. Hence, there

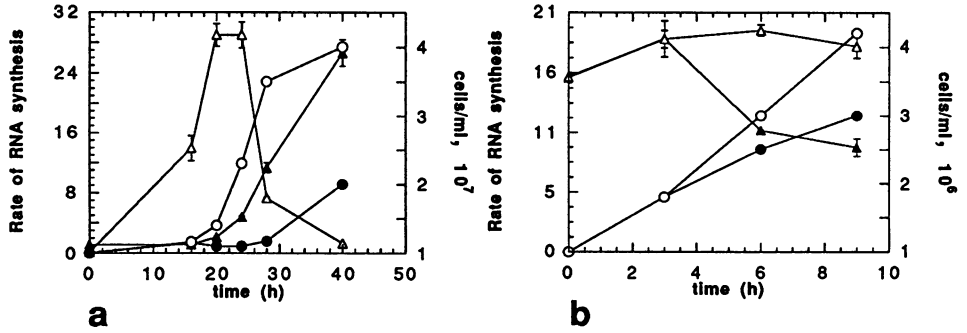

FIG. 7. Rates of transcription and H1 expression. YCL7 and a control strain, YM262/YEp352, were grown in SD to 10^7 cells per ml and shifted to SG (a). Alternatively, galactose was added to both strains grown in glycerol-lactate to 10^6 cells per ml (b). Growth was monitored by cell counting (cells per milliliter; ●, YCL7; ○, YM262/YEp352). Transcription rates were assessed in triplicate by measuring the incorporation of [^{3}H]UTP into RNA (10-min pulses) and counting the TCA-precipitable radioactivity (rates are reported as thousands of counts per minute for 10-min pulses; ▲, YCL7; △, YM262/YEp352).

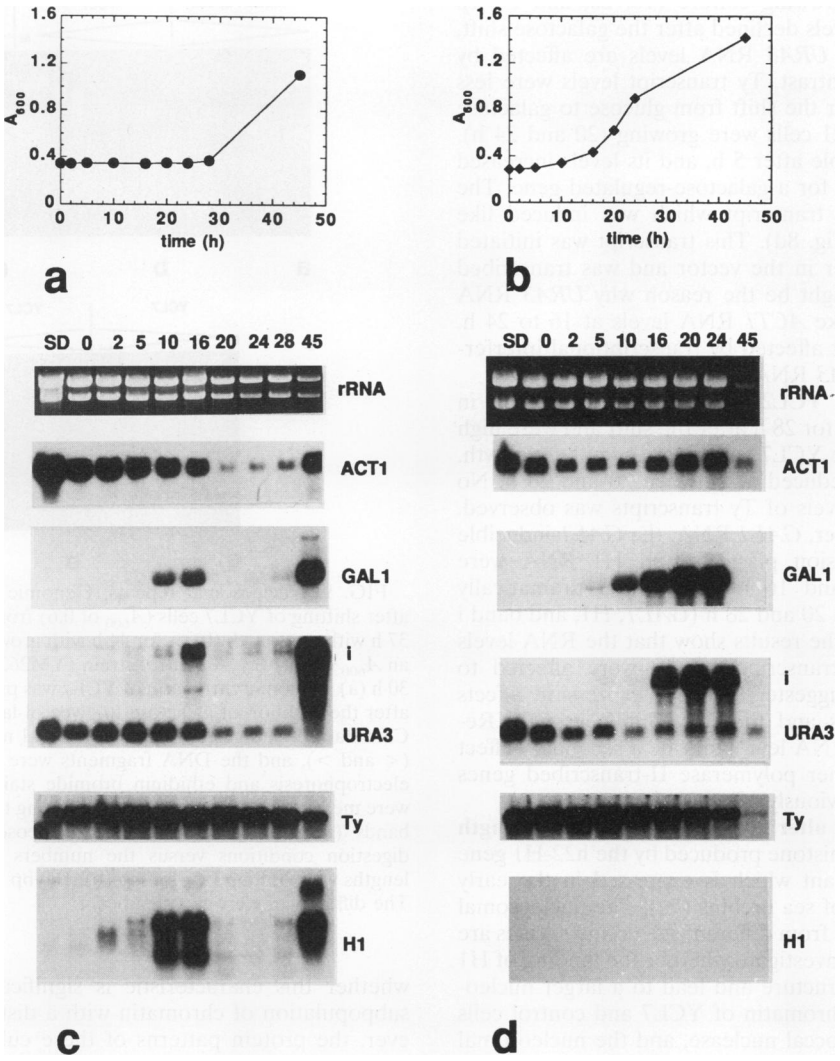

FIG. 8. Northern analysis. YCL7 and control strain YCL21 were grown in SD to an A_{600} of 0.3 and shifted to SG. Growth was monitored (a and b), and RNA was extracted at the times indicated (0, 2, 5, 10, 16, 20, 24, 28, and 45 h). RNA extracted from a fixed number of cells (2×10^7) was loaded per slot, fractionated in agarose, transferred to membranes, and hybridized to various probes (*ACT1*, *URA3*, *GAL1*, Ty elements, and H1). To estimate correct extraction of RNA and loading, coextracted plasmid DNA which hybridized to the *URA3* probe was quantified with a PhosphorImager (for YCL7, in arbitrary units: 1,230 [SD], 1,210 [0 h], 1,142 [2 h], 945 [5 h], 951 [10 h], 875 [16 h], 1,364 [20 h], 1,524 [24 h], 1,075 [28 h], and 275 [45 h]). Autoradiograms are shown for YCL7 (c) and YCL21 (d). The coextracted rRNA was visualized by staining with ethidium bromide (rRNA). Transcripts induced by the *GAL1* promoter and transcribed through *URA3* on the plasmids are indicated by "i".

was a correlation between growth and RNA synthesis. It is possible that H1 expression affects transcription by binding to chromatin and thereby affects growth.

Since [^{3}H]uracil incorporation into cold TCA-precipitable material measures primarily rRNA synthesis by RNA polymerase I (approximately 80%) and only 20% of RNA polymerase II transcription (42), Northern (RNA) blot analysis was used to monitor the fate of a few transcripts produced by RNA polymerase II (Fig. 8). RNAs of *ACT1*, Ty elements, and a galactose-inducible gene (*GAL1*) were selected as chromosomal transcripts, and *URA3* RNA was tested as the product of the marker gene of the expression vector and control plasmid. In these experiments, strain YCL7 was compared with strain YCL21, which contains the control plasmid with the *GAL1-GAL10* promoter. The strains were shifted from glucose to galactose as indicated in the legend to Fig. 8. While YCL21 resumed growth at between 10 and 16 h after the shift, YCL7 remained blocked for over 30 h, as described above (Fig. 8a and b). RNA was extracted and analyzed at various times after the shift. RNA of a fixed number of cells was loaded on gels. The efficiency of extraction and loading was checked by quantification of coextracted plasmid DNA with a PhosphorImager (as indicated in the legend to Fig. 8). Plasmid DNA hybridizes with the *URA3* probe. With the exception of that for stationary-phase cells (45 h), extraction efficiencies were similar.

Ethidium bromide staining showed reduced levels of rRNA in YCL7 after 20 h of induction. This result is consistent with the reduced rRNA synthesis inferred from the [^{3}H]uracil incorporation experiments (Fig. 7).

While in YCL21 high levels of *ACT1* RNA were present in glucose-containing medium (Fig. 8d, lane SD), and immediately after the shift to galactose-containing medium (lane 0), *ACT1* RNA levels declined for about 10 h after the shift and

increased again in the growing culture (16, 20, and 24 h). Similarly, *URA3* RNA levels declined after the galactose shift, showing that *ACT1* and *URA3* RNA levels are affected by growth conditions. In contrast, Ty transcript levels were less affected immediately after the shift from glucose to galactose but declined when YCL21 cells were growing (20 and 24 h). *GAL1* RNA was detectable after 5 h, and its levels increased dramatically, as expected for a galactose-regulated gene. The *URA3* probe detected a transcript which was induced like *GAL1* RNA (band i in Fig. 8d). This transcript was initiated from the *GAL1* promoter in the vector and was transcribed through *URA3*, which might be the reason why *URA3* RNA levels did not increase like *ACT1* RNA levels at 16 to 24 h. However, growth was not affected by transcriptional interference or by antisense *URA3* RNA.

In contrast to those in YCL21 cells, *ACT1* RNA levels in YCL7 cells remained low for 28 h after the shift and were high again after 45 h, when the YCL7 culture resumed late growth. *URA3* levels were also reduced at between 20 and 28 h. No dramatic effect on the levels of Ty transcripts was observed. Most interestingly, however, *GAL1* RNA, the *GAL1*-inducible transcript of the expression plasmid, and H1 RNA were induced at between 5 and 16 h but showed dramatically reduced levels at between 20 and 28 h (*GAL1*, H1, and band i in Fig. 8c). In summary, the results show that the RNA levels of some polymerase II-transcribed genes were affected to different extents. It is suggested that H1 expression affects galactose-regulated genes and thereby inhibits growth. Reduced *ACT1* and *URA3* RNA levels may be a secondary effect of growth inhibition. Other polymerase II-transcribed genes (Ty elements) are not obviously affected.

H1 expression does not alter the nucleosomal repeat length in *S. cerevisiae*. The H1α histone produced by the h22-H1 gene represents a histone variant which is expressed in the early development (blastulas) of sea urchins (7a). The nucleosomal repeat lengths in blastulas from different sea urchin species are about 213 bp (2, 44). To investigate whether the binding of H1 might affect chromatin structure and lead to a larger nucleosome spacing, genomic chromatin of YCL7 and control cells was digested with micrococcal nuclease, and the nucleosomal DNA was analyzed by gel electrophoresis (Fig. 9). YCL7 cells that were incubated for 37 h after a shift from glucose to galactose and did not grow had a nucleosomal repeat pattern similar to that of control cells grown in galactose (YM262/YEp352; compare Fig. 9a and b). The repeat lengths were about 160 bp, well within the range of previous observations (7, 30, 59). The differences in repeat lengths indicated in the legend to Fig. 9 were not significant and were related to differences in gels and digestion conditions. Most importantly, there was no indication of a longer repeat length. In that preparation (Fig. 9b), 83% of the cells survived the galactose treatment and 82% of the surviving cells maintained the expression plasmid.

After longer incubation times in galactose, YCL7 cells started to grow slowly but showed reduced survival and a loss of the expression plasmid. To test whether such a growing culture might reveal a change in repeat length by replication of chromatin, YCL7 cells were shifted from glucose to galactose at an A_{600} of 0.5, and a slowly growing culture was harvested after 44 h, at an A_{600} of 1.1. In this particular experiment, 60% of the cells survived the galactose treatment and 50% of the surviving cells lost the plasmid. Chromatin analysis did not reveal a change in the nucleosomal repeat length or additional bands that would indicate the presence of a different repeat in a subpopulation of the culture (Fig. 9c). Although the repeat pattern was somewhat more smeared, we do not yet know whether this characteristic is significant and may reflect a subpopulation of chromatin with a disturbed structure. However, the protein patterns of these cultures did not reveal a significant degradation of histone proteins (data not shown), indicating that at the time of harvest, chromatin was essentially intact.

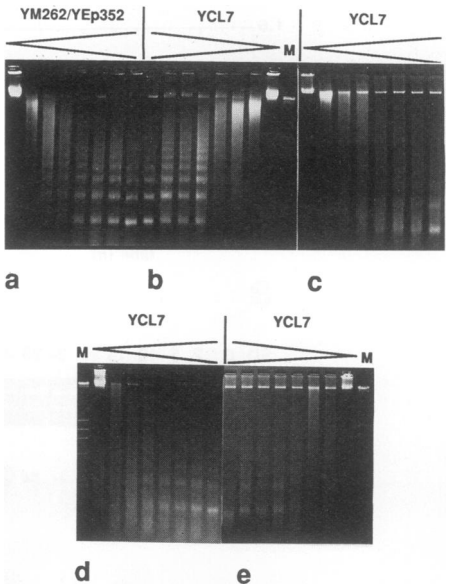

FIG. 9. Nucleosomal repeats. Genomic chromatin was prepared after shifting of YCL7 cells (A_{600} of 0.6) from glucose to galactose for 37 h without growth (b) or for 44 h with growth (from an A_{600} of 0.5 to an A_{600} of 1.1) (c). A control strain (YM262/YEp352) was shifted for 30 h (a). Genomic chromatin of YCL7 was prepared 0 h (d) and 6 h (e) after the addition of galactose to glycerol-lactate-containing medium. Chromatin was digested with micrococcal nuclease at different levels (< and >), and the DNA fragments were visualized by agarose gel electrophoresis and ethidium bromide staining. The repeat lengths were measured on separate gels by plotting the lengths of nucleosomal bands (mononucleosomes to hexanucleosomes) averaged over all digestion conditions versus the numbers of nucleosomes. Repeat lengths were 158 bp (a), 153 bp (b), 160 bp (c and d), and 163 bp (e). The differences were not significant.

To further test whether some growth during H1 expression might disturb the overall chromatin structure, YCL7 cultures were grown in glycerol-lactate and induced in glycerol-lactate plus galactose for 6 h (Fig. 9 d and e). During incubation in galactose, the cultures grew from an A_{600} of 0.25 to an A_{600} of 0.5. The fraction of cells surviving the galactose treatment dropped to 40%, and only 55% of the surviving cells maintained the expression plasmid. For the control chromatin preparation in glycerol-lactate (Fig. 9d), the cultures were harvested at an A_{600} of 0.5. These cultures had a survival rate of 70%, and 86% of the surviving cells contained the expression plasmid. Despite the slow growth in galactose, no obvious disruption of the nucleosomal repeat pattern or an altered repeat length was observed, confirming the observations made when cells were shifted from glucose to galactose.

Expression of the C-terminal tail of histone H1. Deletion of a *Bam*HI fragment of YEpCL11 leads to a construct, YEpCL13, that promotes the expression of amino acids 89 to 206 of h22-H1 (Fig. 10a). This peptide contains one end of the globular domain and the whole C-terminal tail (62). YEpCL13 was used to transform YM262 to yield YCL22. When expression was induced on selective media containing galactose, the C-terminal tail of H1 was detected as an additional protein in

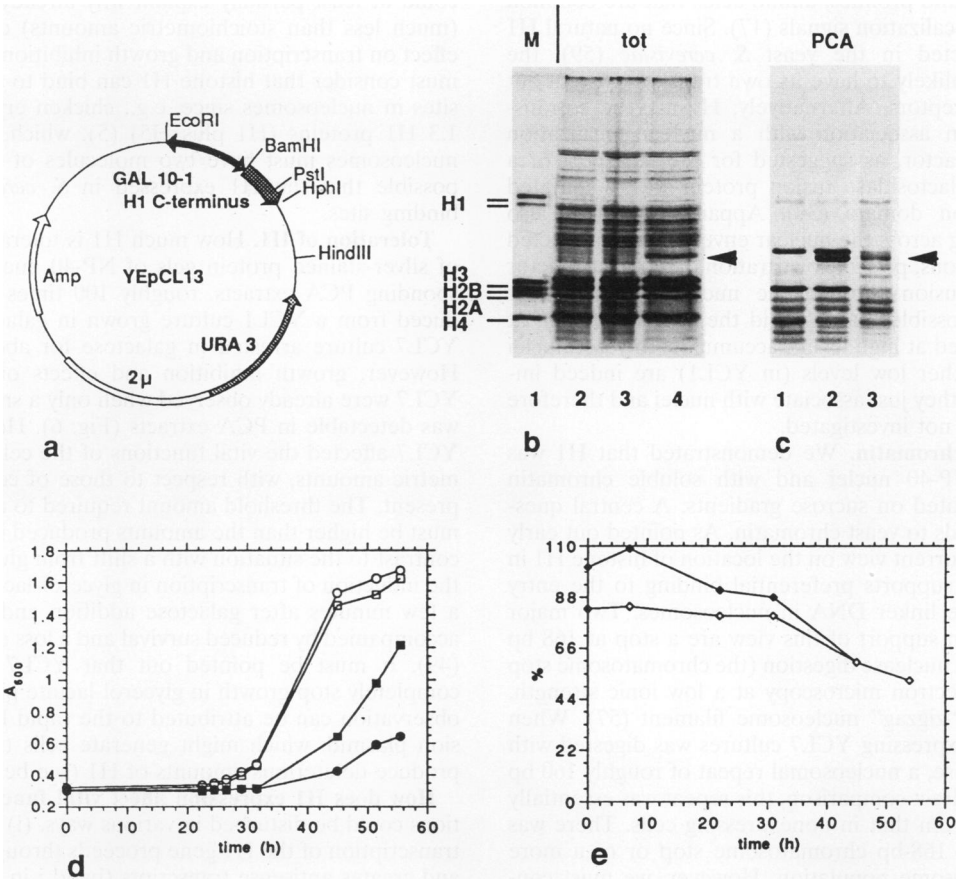

FIG. 10. Expression of the C-terminal tail of H1. (a) YEpCL13 allows the expression of the C-terminal tail (amino acids 89 to 206). (b) Total proteins of NP-40 nuclei were prepared from cells grown in galactose for 42 h and visualized by SDS-polyacrylamide gel electrophoresis and silver staining (tot). Strains tested were YCL22 (containing YEpCL13) (lane 4), YM262/YEp352 (lane 2), and YCL21 (lane 3). Lanes contained approximately 1.4 μg of DNA (lanes 2, 3, and 4) and 0.75 μg of DNA (lane 1; rat liver histone marker [M]). The arrowheads indicate the position of the C-terminal tail. (c) PCA (5%) extracts of NP-40 nuclei of YCL22 cells grown in galactose for 22 (lane 1), 31 (lane 2), and 45 (lane 3) h. (d) Growth curves for cultures after a shift from glucose to galactose. Symbols: ■, YCL22; ●, YCL7; ○, YM262/YEp352; □, YCL21. (e) Survival (◆) and plasmid stability (◇) of YCL22 after a shift from glucose to galactose.

NP-40 nuclei (Fig. 10b). It was soluble in 5% PCA extracts of NP-40 nuclei and showed an apparent molecular weight comparable to that of the C-terminal tail of rat liver histone H1 (58) or sea urchin sperm histone H1 (19). The C-terminal tail was expressed in amounts roughly similar to those of core histones. Expression of the C-terminal tail correlated with growth inhibition on plates (data not shown) and in liquid media, with reduced survival and increased plasmid loss (Fig. 10d and e). These effects, however, were weaker than the effects on the expression of whole H1.

DISCUSSION

Recently, the expression of *Xenopus laevis* histone H5 in *S. cerevisiae* was shown to yield a product which was proteolytically processed (27% smaller), and no effect on growth was observed (46). In contrast to that report, we show that the histone H1 gene of the sea urchin early histone gene cluster could be expressed in *S. cerevisiae* at different levels. The protein had the same apparent molecular weight as the corresponding protein in sea urchins (H1α; 7a) and, hence, was not significantly degraded, except in old cultures. We show that H1 was copurified with chromatin fragments solubilized from NP-40 nuclei and conclude that H1 was bound to chromatin. Furthermore, effects on growth as well as effects on transcription, survival, and plasmid loss correlated with the expression of relatively large amounts of H1, while low levels of H1 were tolerated. Expression of the C-terminal tail had similar effects, but at a reduced level. We infer that the effects are related to histone H1 binding to chromatin, an inference that opens several questions.

Localization of H1 in nuclei. How does H1 or the C-terminal tail end up in nuclei? Although the possibility that H1 and the C-terminal tail were associated with chromatin during lysis of spheroplasts and not in living cells cannot be formally excluded, NP-40 washes, which were used to remove potential contaminants, such as cytoplasmic material and ribosomes (29), did not remove the expressed proteins. In addition, the correlation of H1 expression at high levels with effects on growth, transcription, plasmid loss, and survival argue in favor of a direct effect of H1 at the chromatin level. Although histones are small enough to diffuse into nuclei, injection of histone H1 and its carboxy-terminal domain into the cytoplasm of *Xenopus* oocytes or cell cultures suggested that H1 was transported by a receptor-mediated process rather than by passive diffusion (9, 14). No proper nuclear localization signals are known for H1 proteins (9), but H1 and the C-terminal tail

are rich in lysines and prolines, amino acids that are common to many nuclear localization signals (17). Since no natural H1 protein was detected in the yeast *S. cerevisiae* (59), the expressed H1 is unlikely to have its own transport system but may use other receptors. Alternatively, H1 may be cotransported through an association with a nuclear localization signal-containing factor, as suggested for the transport of a histone H2B–β-galactosidase fusion protein with a mutated nuclear localization domain (36). Apparently, H1 is also capable of diffusing across the nuclear envelope when injected in high concentrations, possibly by titration of a limiting factor that prevents diffusion through the nuclear pores (9). It therefore seems possible that H1 and the C-terminal tail, at least when expressed at high levels, accumulate in yeast nuclei by diffusion. Whether low levels (in YCL1) are indeed imported or whether they just associate with nuclei and therefore are copurified was not investigated.

H1 binding to chromatin. We demonstrated that H1 was copurified with NP-40 nuclei and with soluble chromatin fragments fractionated on sucrose gradients. A central question is how H1 binds to yeast chromatin. As pointed out early in this paper, our current view on the location of histone H1 in higher eukaryotes supports preferential binding to the entry and exit site of the linker DNA in nucleosomes. Two major lines of evidence in support of this view are a stop at 168 bp during micrococcal nuclease digestion (the chromatosome stop [1, 38, 47]) and electron microscopy at a low ionic strength, showing an open "zigzag" nucleosome filament (57). When chromatin of H1-expressing YCL7 cultures was digested with micrococcal nuclease, a nucleosomal repeat of roughly 160 bp was observed; by direct comparison, this repeat was essentially indistinguishable from that in nonexpressing cells. There was no indication of a 168-bp chromatosome stop or of a more stable mononucleosome population. However, we must consider that the chromatosome stop in higher eukaryotes is a transient state and can only be observed when the repeat length is significantly longer, e.g., close to 200 bp, as in rat liver or chicken erythrocytes. As long as the yeast repeat is too close to the chromatosome stop, it might be very difficult to detect a subpopulation with a defined 168-bp stop. We also considered the possibility that H1 deposition might affect the kinetics of micrococcal nuclease digestion. However, since this enzyme cuts DNA and RNA and since *S. cerevisiae* contains a large quantity RNA subtle kinetic experiments with this enzyme were not appropriate. On the basis of our experience (55–57), we strongly considered using electron microscopy as an approach for estimating proper H1 binding. However, electron microscopy of soluble chromatin was not the approach for the following reasons. (i) The zigzag phenotype of nucleosome filaments is characteristic of H1-containing chromatin but does not allow quantitation of the correct binding of H1. Because of plasmid loss and time-dependent H1 expression, individual cells of the YCL7 strain might show different amounts of H1, which would result in a very heterologous population of chromatin fragments. Furthermore, the preparation of soluble chromatin fragments contained significant amounts of ribonucleoprotein contamination, and the solubilized fragments were relatively short for electron microscopy and apparently had a lower content of H1 than the NP-40 nuclei (see Results).

Although the average nucleosomal spacing is small, it must be considered that individual nucleosomes can be fairly well spaced, e.g., in the *URA3* and *HIS3* genes; in particular, promoter nucleosomes are flanked by nuclease-sensitive regions which may provide sufficient DNA for making two complete turns (32, 52, 54). Hence, it is possible that H1 correctly binds to a subset of nucleosomes. This possibility could at least partially explain why already low levels of H1 (much less than stoichiometric amounts) correlated with an effect on transcription and growth inhibition. Furthermore, we must consider that histone H1 can bind to additional binding sites in nucleosomes since, e.g., chicken erythrocytes contain 1.3 H1 proteins (H1 plus H5) (5), which means that some nucleosomes must have two molecules of H1 proteins. It is possible that the H1 expressed in *S. cerevisiae* uses these binding sites.

Toleration of H1. How much H1 is tolerated? On the basis of silver-stained protein gels of NP-40 nuclei and the corresponding PCA extracts, roughly 100 times less H1 was produced from a YCL1 culture grown in galactose than from a YCL7 culture arrested in galactose for about 40 h (Fig. 2). However, growth inhibition and effects on transcription in YCL7 were already observed when only a small amount of H1 was detectable in PCA extracts (Fig. 6). Hence, H1 in strain YCL7 affected the vital functions of the cells before stoichiometric amounts, with respect to those of core histones, were present. The threshold amount required to affect cell function must be higher than the amounts produced in strain YCL1. In contrast to the situation with a shift from glucose to galactose, the induction of transcription in glycerol-lactate started within a few minutes after galactose addition and was immediately accompanied by reduced survival and a loss of plasmid stability (49). It must be pointed out that YCL7 cultures did not completely stop growth in glycerol-lactate plus galactose. This observation can be attributed to the rapid loss of the expression plasmid, which might generate cells that are unable to produce deleterious amounts of H1 (see below).

How does H1 expression affect vital functions? Vital functions could be disturbed in various ways. (i) We observed that transcription of the H1 gene proceeds through the *URA3* gene and creates antisense transcripts (band i in Fig. 8). The same observation, however, was made for control strain YCL21, containing the *GAL1-GAL10* promoter construct. Since YCL21 did not show any effect on growth retardation, we exclude transcriptional interference with the marker gene and antisense RNA as the cause for the H1 expression effects. The data on YCL21 also allow us to exclude the possibility that titration of a limited amount of transcription factors might have hampered galactose metabolism, thereby affecting growth properties and survival.

(ii) H1 represses transcription. We used uracil incorporation into RNA and Northern analysis to study transcription when YCL7 cells were expressing H1 and growth was inhibited. Uracil incorporation measures primarily rRNA synthesis (approximately 80%) and only about 20% of polymerase II transcription (42). We observed reduced uracil incorporation into RNA (Fig. 7) and reduced rRNA levels (Fig. 8), suggesting that H1 might repress transcription. RNA analysis of a few selected genes transcribed by RNA polymerase II allowed a more detailed interpretation. While the *ACT1* and *URA3* RNA levels were reduced, Ty RNA levels were not significantly reduced, indicating that not all genes were equally affected. This observation argues against a general repression of all genes by histone H1. Most dramatically, however, the levels of the galactose-inducible transcripts of the chromosomal *GAL1* gene and of the expression plasmid (H1, band i in Fig. 8) were transiently elevated (approximately 5 to 16 h after the shift), as expected from control constructs, but were greatly reduced after 16 h. This observation suggests that H1 represses the transcription of galactose-regulated genes. Since the *ACT1* and *URA3* RNA levels in control cells were also reduced after a shift from glucose to galactose, demonstrating growth dependence, we infer that the reduced *ACT1* and *URA3* RNA levels

could be a consequence of growth inhibition. Alternatively, a rather impressive drop in *ACT1* RNA, *URA3* RNA, and even rRNA levels between 16 and 24 h would also be consistent with repression by H1.

The mechanism of the H1 effect on transcription of the galactose-regulated genes is not known. One possibility is a selective repression of galactose-inducible genes by H1 binding to chromatin, e.g., in the promoter region. This region is particularly sensitive, since histones and nucleosomes play an active role in its transcriptional regulation (reviewed in references 18 and 34) and a number of studies reported changes in the chromatin structure of the *GAL1* promoter upon activation (11, 15, 28, 31). It was reported that GAL4, the activator of transcription in the *GAL1* promoter, may disrupt nucleosome structure in vitro and in vivo (3, 63) and that a GAL4-VP16 fusion protein can relieve the repression of transcription by histone H1 in vitro (12, 24). Hence, it is possible that H1 binding stabilizes the *GAL1* nucleosome and that GAL4 is not sufficient to overcome H1 repression. Promoter studies will be performed to analyze this topic.

If one mode of action involves binding to regulatory nucleosomes, we assume that genes which are not regulated by nucleosomes will be less stringently affected by H1. This could be one explanation for the differences between the tested RNAs. An alternative mode of action for histone H1 is general repression by chromatin condensation, which would affect initiation and elongation of transcription. This explanation is not favored, since chromatin condensation in vitro requires substantial amounts of H1 (57, 58), but only a small amount of H1 was detected when growth was inhibited and the galactose-regulated genes were repressed. Furthermore, we would expect that general repression by chromatin condensation would also substantially affect Ty RNA levels.

(iii) Although these results for transcription are consistent with the hypothesis that H1 binding to chromatin inhibits the transcription of some genes, we cannot exclude the possibility that H1 affects vital functions by interfering with some other pathways, such as galactose metabolism and RNA transport, processing, or eventually translation. A putative exhausting of the translation system seems unlikely, since effects were already observed before maximal amounts of H1 were produced. In particular, we do not have a good explanation for the fact that large amounts of H1 RNA and H1 protein were produced after 45 h. At this point, however, the cell population was heterogeneous because of the increased plasmid loss (see below), which complicates a more detailed analysis.

(iv) Additional information on how H1 affects vital functions may be inferred from the expression of its C-terminal tail, which had similar effects, but at a lower level. The highly charged C-terminal tail most efficiently induces condensation and precipitation of chromatin in vitro (58), while the globular domain is required for binding to the nucleosome at the entry and exit site (1). Hence, at least some of the H1 effects might be explained by nonspecific binding and local condensation of chromatin. Whether the rest involve the globular domain and the N-terminal tail as well as specific binding to the nucleosome needs to be studied.

H1 expression and plasmid stability. The expression of H5 in addition to endogenous H1 affected cell proliferation (51). Although we observed strong growth retardation in galactose, the YCL7 strain did not show defined cell division cycle arrest (data not shown). In particular, we could not find any indication of a block during mitosis, which would indicate a problem with chromosome segregation. However, cells that survived H1 expression showed a dramatic increase in the loss of the expression plasmid, indicating that the plasmid replication and maintenance system was disturbed. One possibility is that transcription initiating at the *GAL10* promoter on the plasmid interferes with elements important for chromosome maintenance (48). This possibility could be excluded, since strain YCL21, carrying a control plasmid with the same promoter but with a deletion of the H1 gene, showed normal growth and no increase in plasmid loss. An alternative explanation is that binding of histone H1 and condensation of the expression plasmid might prevent assembly of the replication machinery or hamper replication or segregation, since under conditions of plasmid loss, both in glucose-galactose shift experiments and in glycerol-lactate–galactose shift experiments, large amounts of H1 were detected.

We observed that after long incubation times on plates or in liquid cultures, YCL7 started to grow (slowly growing colonies). The number of colonies on plates was much too high to attribute this effect to mutations in the H1 expression system. One explanation which we favor is as follows. Since plasmid loss is high, cells with low expression plasmid copy numbers must occasionally exist. Since low levels of H1 expression are tolerated (e.g., in YCL1), these slowly growing colonies must originate from cells that maintain a low expression plasmid copy number.

H1 and the nucleosomal repeat. Despite the expression of roughly stoichiometric amounts of H1 in the chromatin of YCL7 cells, the nucleosomal repeat length was not changed. This result complements the observations made with H5 overexpression in rat sarcoma cells (50). Hence, simple deposition of H1 was not sufficient to establish an altered repeat. In contrast to rat cells, the yeast *S. cerevisiae* has a very short nucleosomal repeat length (about 160 bp) (7, 30, 59), the DNA is almost saturated with histones, and no space is available to generate a longer linker DNA. Hence, such a mechanism would require either the dissociation of core histones or an additional round of replication. However, we did not observe an altered nucleosomal repeat when cell cultures were slowly growing in galactose. This result can be explained by the heterogeneity of the growing population. Since only low levels of H1 were tolerated and a high level of plasmid loss was apparent under these conditions, we assume that only the particular fraction of cells that lost or partially lost the expression plasmid contributed to growth. These cells, however, would not have produced sufficient H1 to alter the inherited repeat pattern.

ACKNOWLEDGMENTS

We thank R. Davis, D. Gallwitz, M. Johnston, and P. Philippsen for gifts of plasmids and yeast strains, M. L. Birnstiel and M. Busslinger for the h22 DNA, the DNA sequence, and the sea urchin histones, and D. Doenecke for communication of unpublished results. We also thank E. Furter, S. Tanaka, and G. Cavalli for comments on the manuscript and T. Koller for continous support.

This work was supported by grants to F.T. from the Swiss National Science Foundation and the ETH-Zürich.

REFERENCES

1. **Allan, J., P. G. Hartmann, C. Crane-Robinson, and F. X. Aviles.** 1980. The structure of histone H1 and its location in chromatin. Nature (London) **288**:675–679.
2. **Arceci, R. J., and P. R. Gross.** 1980. Histone variants and chromatin structure during sea urchin development. Dev. Biol. **80**:186–209.
3. **Axelrod, J. D., M. S. Reagan, and J. Majors.** 1993. GAL4 disrupts a repressing nucleosome during activation of GAL1 transcription in vivo. Genes Dev. **7**:857–869.
4. **Bates, D. L., P. J. G. Butler, E. C. Pearson, and J. O. Thomas.** 1981. Stability of the higher order structure of chicken erythrocyte

chromatin in solution. Eur. J. Biochem. **119**:469–476.
5. **Bates, D. L., and J. O. Thomas.** 1981. Histones H1 and H5: one or two molecules per nucleosome? Nucleic Acids Res. **9**:5883–5894.
6. **Bavykin, S. G., S. I. Usachenko, A. I. Lishanskaya, V. V. Shick, A. V. Belyavsky, I. M. Undritsov, A. A. Strokov, I. A. Zalenskaya, and A. D. Mirzabekov.** 1985. Primary organization of nucleosomal core particles is invariable in repressed and active nuclei from animal, plant and yeast cells. Nucleic Acids Res. **13**:3439–3459.
7. **Bernardi, F., T. Koller, and F. Thoma.** 1991. The ade6 gene of the fission yeast Schizosaccharomyces pombe has the same chromatin structure in the chromosome and in plasmids. Yeast **7**:547–558.
7a. **Birnstiel, M. L., and M. Busslinger (Institute of Molecular Pathology, Vienna, Austria).** Personal communication.
8. **Boulikas, T., J. M. Wiseman, and W. T. Garrard.** 1980. Points of contact between histone H1 and the histone octamer. Proc. Natl. Acad. Sci. USA **77**:127–131.
9. **Breeuwer, M., and D. S. Goldfarb.** 1990. Facilitated nuclear transport of histone H1 and other small nucleophilic proteins. Cell **60**:999–1008.
10. **Brill, S. J., S. DiNardo, K. Voelkel-Meiman, and R. Sternglanz.** 1987. Need for DNA topoisomerase activity as a swivel for DNA replication and for transcription of ribosomal RNA. Nature (London) **326**:414–416.
11. **Cavalli, G., and F. Thoma.** 1993. Chromatin transitions during activation and repression of galactose-regulated genes in yeast. EMBO J. **12**:4603–4613.
12. **Croston, G. E., P. J. Laybourn, S. M. Paranjape, and J. T. Kadonaga.** 1992. Mechanism of transcriptional antirepression by GAL4-VP16. Genes Dev. **6**:2270–2281.
13. **Davie, J. R., C. A. Saunders, J. M. Walsh, and S. C. Weber.** 1981. Histone modifications in the yeast *S. cerevisiae*. Nucleic Acids Res. **9**:3205–3215.
14. **Dingwall, C., and J. Allan.** 1984. Accumulation of the isolated carboxy-terminal domain of histone H1 in the Xenopus oocyte nucleus. EMBO J. **3**:1933–1937.
15. **Fedor, M. J., and R. D. Kornberg.** 1989. Upstream activation sequence-dependent alteration of chromatin structure and transcription activation of the yeast *GAL1-GAL10* genes. Mol. Cell. Biol. **9**:1721–1732.
16. **Frado, L.-L. Y., C. V. Mura, B. D. Stollar, and C. L. F. Woodcock.** 1983. Mapping of histone H5 sites on nucleosomes using immunoelectron microscopy. J. Biol. Chem. **258**:11984–11990.
17. **Gerace, L., and B. Burke.** 1988. Functional organization of the nuclear envelope. Annu. Rev. Cell Biol. **4**:335–374.
18. **Grunstein, M.** 1990. Nucleosomes: regulators of transcription. Trends Genet. **6**:395–400.
19. **Hill, C. S., S. R. Martin, and J. O. Thomas.** 1989. A stable α-helical element in the carboxy-terminal domain of free and chromatin-bound histone H1 from sea urchin sperm. EMBO J. **8**:2591–2599.
20. **Hill, J. E., A. M. Myers, T. J. Koerner, and A. Tzagoloff.** 1986. Yeast/E. coli shuttle vectors with multiple unique restriction sites. Yeast **2**:163–167.
21. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
22. **Jensen, R., G. F. Sprague, and I. Herskowitz.** 1983. Regulation of yeast mating-type interconversion: feedback of HO gene expression by the mating-type. Proc. Natl. Acad. Sci. USA **80**:3035–3039.
23. **Johnston, M., and R. W. Davis.** 1984. Sequences that regulate the divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:1440–1448.
24. **Kamakaka, R. T., M. Bulger, and J. T. Kadonaga.** 1993. Potentiation of RNA polymerase-II transcription by Gal4-VP16 during but not after DNA replication and chromatin assembly. Genes Dev. **7**:1779–1795.
25. **Künzler, P., and A. Stein.** 1983. Histone H5 can increase the internucleosomal spacing in dinucleosomes to nativelike values. Biochemistry **22**:1783–1789.
26. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
27. **Lennard, A. C., and J. O. Thomas.** 1985. The arrangement of H5 molecules in extended and condensed chicken erythrocyte chromatin. EMBO J. **4**:3455–3462.
28. **Lohr, D.** 1984. Organization of the GAL1-GAL10 intergenic control region chromatin. Nucleic Acids Res. **12**:8457–8474.
29. **Lohr, D.** 1988. Isolation of yeast nuclei and chromatin for studies of transcription-related processes, p. 125–145. *In* I. Campbell and J. H. Duffus (ed.), Yeast: a practical approach. IRL Press, Oxford.
30. **Lohr, D., R. T. Kovacic, and K. E. Van Holde.** 1977. Quantitative analysis of yeast chromatin by staphylococcal nuclease. Biochemistry **16**:463–471.
31. **Lohr, D., T. Torchia, and J. Hopper.** 1987. The regulatory protein GAL80 is a determinant of the chromatin structure of the yeast GAL1-10 control region. J. Biol. Chem. **262**:15589–15597.
32. **Losa, R., S. Omari, and F. Thoma.** 1990. Poly(dA)·poly(dT) rich sequences are not sufficient to exclude nucleosome formation in a constitutive yeast promoter. Nucleic Acids Res. **18**:3495–3502.
33. **Losa, R., F. Thoma, and T. Koller.** 1984. Involvement of the globular domain of histone H1 in the higher order structures of chromatin. J. Mol. Biol. **175**:529–551.
34. **Mann, R. K., and M. Grunstein.** 1992. Histone H3 N-terminal mutations allow hyperactivation of the yeast GAL1 gene in vivo. EMBO J. **11**:3297–3306.
35. **Meeks-Wagner, D., and L. H. Hartwell.** 1986. Normal stoichiometry of histone dimer sets is necessary for high fidelity of mitotic chromosome transmission. Cell **44**:43–52.
36. **Moreland, R. B., G. L. Langevin, R. H. Singer, R. L. Garcea, and L. M. Hereford.** 1987. Amino acid sequences that determine the nuclear localization of yeast histone 2B. Mol. Cell. Biol. **7**:4048–4057.
37. **Neelin, J. M., P. X. Callahan, D. C. Lamb, and K. Murray.** 1964. The histones of chicken erythrocyte nuclei. Can. J. Biochem. **42**:1743–1752.
37a. **Negri, R.** Personal communication.
38. **Noll, M., and R. D. Kornberg.** 1977. Action of micrococcal nuclease on chromatin and the location of histone H1. J. Mol. Biol. **109**:393–404.
39. **Pederson, D. S., F. Thoma, and R. T. Simpson.** 1986. Core particle, fiber and transcriptionally active chromatin structure. Annu. Rev. Cell Biol. **2**:117–147.
40. **Renz, M., P. Nehls, and J. Hozier.** 1977. Involvement of histone H1 in the organization of the chromosome fiber. Proc. Natl. Acad. Sci. USA **74**:1879–1883.
41. **Sanders, C., and E. W. Johns.** 1974. A method for the large-scale preparation of two chromatin proteins. Biochem. Soc. Trans. **2**:547–550.
42. **Santiago, T. C., I. J. Purvis, A. J. E. Bettany, and A. J. P. Brown.** 1986. The relationship between mRNA stability and length in *Saccharomyces cerevisiae*. Nucleic Acids Res. **14**:8347–8360.
43. **Schaffner, W., G. Kunz, H. Daetwyler, J. Telford, H. O. Smith, and M. L. Birnstiel.** 1978. Genes and spacers of cloned sea urchin histone DNA analyzed by sequencing. Cell **14**:655–671.
44. **Shaw, B. R., G. Cognetti, W. M. Sholes, and R. G. Richards.** 1981. Shift in nucleosome populations during embryogenesis: microheterogeneity in nucleosomes during development of the sea urchin embryo. Biochemistry **20**:4971–4978.
45. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1981. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
46. **Shwed, P. S., J. M. Neelin, and V. L. Seligy.** 1992. Expression of Xenopus laevis histone H5 gene in yeast. Biochim. Biophys. Acta **1131**:152–160.
47. **Simpson, R. T.** 1978. Structure of the chromatosome, a chromatin particle containing 160 base pairs of DNA and all the histones. Biochemistry **17**:5524–5531.
48. **Snyder, M., R. Sapolsky, and R. W. Davis.** 1988. Transcription interferes with elements important for chromosome maintenance in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8**:2184–2194.
49. **St. John, T. P., and R. W. Davis.** 1981. The organization and transcription of the galactose gene cluster of *Saccharomyces*. J. Mol. Biol. **152**:285–315.
50. **Sun, J.-M., Z. Ali, R. Lurz, and A. Ruiz-Carrillo.** 1990. Replacement of histone H1 by H5 in vivo does not change the nucleosome

repeat length of chromatin but increases its stability. EMBO J. **9**:1651–1658.
51. **Sun, J.-M., R. Wiaderkiewicz, and A. Ruiz-Carrillo.** 1989. Histone H5 in the control of DNA synthesis and cell proliferation. Science **245**:68–71.
52. **Thoma, F.** 1986. Protein-DNA interactions and nuclease sensitive regions determine nucleosome positions on yeast plasmid chromatin. J. Mol. Biol. **190**:177–190.
53. **Thoma, F.** 1988. The role of histone H1 in nucleosomes and chromatin fibers, p. 163–185. *In* G. Kahl (ed.), Architecture of eukaryotic genes. VCH, Weinheim, Germany.
54. **Thoma, F., L. W. Bergman, and R. T. Simpson.** 1984. Nuclease digestion of circular TRP1ARS1 chromatin reveals positioned nucleosomes separated by nuclease sensitive regions. J. Mol. Biol. **177**:715–733.
55. **Thoma, F., and T. Koller.** 1977. Influence of histone H1 on chromatin structure. Cell **12**:101–107.
56. **Thoma, F., and T. Koller.** 1981. Unravelled nucleosomes, nucleosome beads and higher order structures of chromatin: influence of non-histone components and histone H1. J. Mol. Biol. **148**:709–733.
57. **Thoma, F., T. Koller, and A. Klug.** 1979. Involvement of histone H1 in the organization of the nucleosome and of the salt dependent superstructures of chromatin. J. Cell Biol. **83**:403–427.
58. **Thoma, F., R. Losa, and T. Koller.** 1983. Involvement of the domains of histone H1 in the higher order structures of chromatin. J. Mol. Biol. **167**:619–640.
59. **Thomas, J. O., and V. Furber.** 1976. Yeast chromatin structure. FEBS Lett. **66**:274–280.
60. **Thomas, P. S.** 1983. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Methods Enzymol. **100**:254–266.
61. **Tremethick, D. J., and M. Frommer.** 1992. Partial purification, from Xenopus laevis oocytes, of an ATP-dependent activity required for nucleosome spacing in vitro. J. Biol. Chem. **267**:15041–15048.
62. **Van Holde, K. E.** 1989. Chromatin. Springer-Verlag KG, Berlin.
63. **Workman, J. L., and R. E. Kingston.** 1992. Nucleosome core displacement in vitro via a metastable transcription factor nucleosome complex. Science **258**:1780–1784.
64. **Wray, W., T. Boulikas, V. P. Wray, and R. Hancock.** 1981. Silver staining of proteins in polyacrylamide gels. Anal. Biochem. **118**: 197–203.