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Low levels of exogenous histone H1 in yeast cause cell death

(heterologous expression/*Saccharomyces cerevisiae*/electron microscopy/immunoelectron microscopy)

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ABSTRACT **To elucidate the function of lysine-rich histone, yeast cells, which are believed to lack this histone, were transformed with an expression vector carrying the sea urchin histone H1 gene under control of an inducible promoter. Expression of full-length protein was tested by immunoblotting and the intracellular distribution was monitored by immunoelectron microscopy. Even low amounts of exogenous H1 led to dramatic changes in intracellular morphology and cell death. The cells that survived had lost either the plasmid or the ability to express the exogenous protein. Thus, even low amounts of canonical histone H1 are lethal to yeast cells.**

Histone H1 is known to bind to the linker DNA between nucleosomes, sealing off two turns of DNA around the histone octamer (1, 2). It is also involved in the formation and maintenance of higher-order chromatin structures and inhibits transcription by an as yet unknown mechanism (3, 4). One possible approach to elucidate the function of this histone would be to introduce exogenous H1 into appropriate living cells and follow its effects on the cellular function of interest. Yeast seems to be the obvious choice for such experiments, for it is believed not to contain an endogenous typical H1. Although immunological evidence for H1-like proteins in yeast has been reported (5–7), such molecules might be quite different from the linker histones of higher eukaryotes. Moreover, the putative yeast H1 homologue is probably present in very low amounts and may be predominantly or exclusively localized in heterochromatic regions or at telomeres.

Recently, we (8, 9) and others (10) have reported experiments on yeast transformation with expression vectors carrying the gene for sea urchin histone H1 under the control of the inducible *GAL1–GAL10* promoter. The expression of the gene was proven at both the mRNA and the protein level. Here we show that expression of even low amounts of exogenous H1 causes dramatic changes in cellular morphology and leads to cell death.

MATERIALS AND METHODS

Construction of the Multicopy Expression Vector and Yeast Transformation. A 1397-bp *Hpa* I–*Hin*dIII fragment containing the entire sea urchin (*Psammechinis miliaris*) histone H1 gene was obtained from plasmid pUC8h22 (11) and inserted at the *Sal* I–*Hin*dIII site of the polylinker of the shuttle expression vector YEp51 (12) (Fig. 1*A*). In this construct the H1 gene is under the transcriptional control of the *GAL10* promoter sequences and the termination site(s) in the 2-μm yeast plasmid. For further details of the cloning, see ref. 8. The expression vector was introduced into an appropriately constructed yeast strain (8) by the lithium acetate/polyethylene glycol method (13). Selection was performed in minimal medium lacking leucine. Histone H1 gene expression was induced by addition of 2% galactose to cells cultured in lactate (14).

Growth Curves and Colony-Forming-Unit Test. Growth in liquid cultures was recorded as a function of time by measuring turbidity of the culture at 660 nm. Survival was monitored by plating a given number of cells on YPD plates (15), incubating for 3 days, and counting the number of colonies formed.

Western Blotting. Total yeast proteins or 5% perchloric acid-extracted cellular proteins were fractionated electrophoretically in SDS-containing polyacrylamide gels (16) and transferred to nitrocellulose paper by semidry blotting in a model IMM-1 apparatus (W.E.P. Company, Seattle) as recommended by the manufacturer. Anti-mouse liver histone H1 antiserum (5) was presaturated with yeast lysate from the host strain by incubating nitrocellulose sheets (4 cm × 7 cm) with the lysate overnight at 4°C and then with 10 ml of anti-H1 antiserum for 3 hr at 37°C. The filter with the adsorbed antibodies was removed and the remaining serum was used for Western blotting, performed according to routine procedures (5). Presaturation of the serum significantly reduced the background immunoreaction. Dilution of the anti-H1 antiserum was 1000-fold, and the enzyme substrate was *o*-phenylenediamine.

Electron Microscopy. Cells were fixed for 5 min in 3.7% formaldehyde and then with 1% glutaraldehyde overnight at room temperature. Fixed cells were embedded in Lowacryl K4M (17) and ultrathin sections were examined in a JEOL JEM 100B electron microscope.

Immunoelectron Microscopy. The immunostaining was performed directly on the microscopic grids at room temperature: ultrathin sections of Lowacryl-embedded fixed cells were incubated for 1 hr with presaturated serum (see above), which was diluted 400-fold in phosphate-buffered saline (PBS), washed in PBS, and incubated with protein A-gold (Sigma; size of the gold particles, 10 nm) for 1 hr. The electron microscopy was as described above.

RESULTS AND DISCUSSION

Yeast Transformation with an Expression Vector Carrying a Sea Urchin Histone H1 Gene. The construction of the multicopy expression vector that carries the sea urchin (*P. miliaris*) histone H1 gene under the control of the galactose-inducible *GAL1–GAL10* promoter (Fig. 1*A*) has been presented (8). An appropriate yeast strain (*MAT***a**, *gal1, his3-11, his3-15, leu2-3, leu2-112*) was constructed (8) and transformed with the expression vector. H1 expression was induced in exponential lactate-grown cultures by addition of galactose to the medium and monitored by Northern and Western blots (Fig. 1*B*). Although expression was from a multicopy plasmid, the amount of H1 that accumulated in our experiments was low and could be detected only by Western

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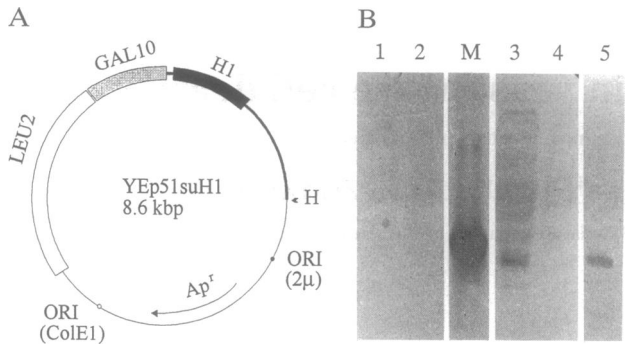

FIG. 1. (*A*) H1 expression vector YEp51suH1. In this construct the H1 gene is under the transcriptional control of the *GAL10* promoter sequences. For further details of the cloning, see ref. 8. ORI, origin; H, transcription termination site. (*B*) Western blots. Total yeast proteins or 5% perchloric acid-extracted cellular proteins were fractionated electrophoretically in SDS-containing polyacrylamide gels, transferred to nitrocellulose paper, and immunoreacted according to routine procedures. Lanes: M, mouse liver histone H1AB used as marker; 1 and 2, lysates from untransformed host on lactate and galactose (12 hr), respectively; 3 and 4, lysates from transformants on galactose (12 hr) and lactate, respectively; 5, 5% perchloric acid-extracted cellular proteins from induced transformant (12 hr in galactose). The amount of protein per lane was such that if H1 were expressed in stoichiometric amounts with respect to the core histones in the induced cultures, it would have given an intense band after Coomassie blue staining. However, no significant difference could be detected by staining between the protein profiles of the noninduced and the induced cells (data not shown). The induced protein could be seen only on the immunoblots. The electrophoretic mobility of the induced protein is slightly higher than that of the mouse markers and corresponds to a full-length sea urchin histone H1 (open reading frame of 206 amino acids, calculated molecular mass 21.7 kDa; ref. 11).

blotting of either total or perchloric acid-extracted proteins at relatively early times of induction, starting from 3 hr (in older cultures the protein was degraded in a cell death-related process; see below). The quick induction was determined by the properties of the induction system used here, in which galactose is added to cells grown in lactate, as opposed to slow induction occurring by a glucose-to-galactose shift (14).

Effect of H1 Expression on Growth and Survival. The effect of H1 expression on growth was initially tested by comparing the increase in cell number with time of culture in liquid selective medium, in the absence or presence of galactose. Fig. 2*A* shows a typical example of such growth curves. Addition of galactose to the medium resulted in considerable inhibition of growth, which was evident for at least 45–50 hr. Upon further incubation the cell density of the two cultures eventually became equal, due to outgrowth of cells not expressing H1 (see below). The growth curves for the host grown in lactose or galactose were similar.

The percentage of cells that survive galactose treatment was determined by the colony-forming-unit test. A given number of cells from control and transformed strains cultivated in the absence or presence of galactose were plated on YPD plates and incubated for 3 days. Survival was determined as percent of the number of colonies formed relative to the number of cells plated. As illustrated in Fig. 2*B*, survival was 100% in the case of the control strain on both carbon sources. For the transformant, survival was 100% on lactate and only 35% and 5% following cultivation on galactose-containing medium for 3 and 12 hr, respectively. Thus, even a low level of H1 expression caused massive cell death. To determine whether the survivors could tolerate H1 expression or survived because they did not express H1, the surviving colonies were further examined for plasmid retention and H1 expression. Fifty percent of the colonies that survived 12 hr in galactose (2.5% of the initial population of transformed cells) had lost the plasmid. A similar high rate of plasmid loss was reported by Linder and Thoma (10) for their high-copy H1 expression vector and was attributed to high levels of H1 expression; results for the low-level expression vector were not reported for the experiments in which induction was performed by shift from glycerol/lactate to galactose.

The remaining 50% of the survivors that retained the plasmid were checked for H1 expression by Western blotting of perchloric acid-extracted cellular proteins. In none of them was H1 detected (data not shown). The lack of expression of H1 might be a consequence of some defect in the expression system, caused, for instance, by recombination events in expression-related regions of the multicopy plasmid or by deficiency of Gal4p, the positive transcription factor for *GAL* genes (14). Thus, the survival data as a whole show that 95% of the cells cultivated in galactose for 12 hr died, presumably as a result of H1 expression, whereas the 5% surviving were viable because they failed to express the protein.

Morphological Changes Caused by H1 Expression. To correlate cell death with changes in intracellular morphology, whole cells were fixed and examined under the electron microscope. The appearance of transformants cultivated on lactate was completely normal (Fig. 3*A*), indistinguishable from that of host cells grown in the same carbon source (data not shown). In accordance with earlier observations (19, 20), the cytoplasm was quite homogeneous, with prominent central vacuole and numerous mitochondria and smaller vesicles clearly visible. The nucleus, somewhat smaller than the central vacuole, was also quite uniformly dense, with occa-

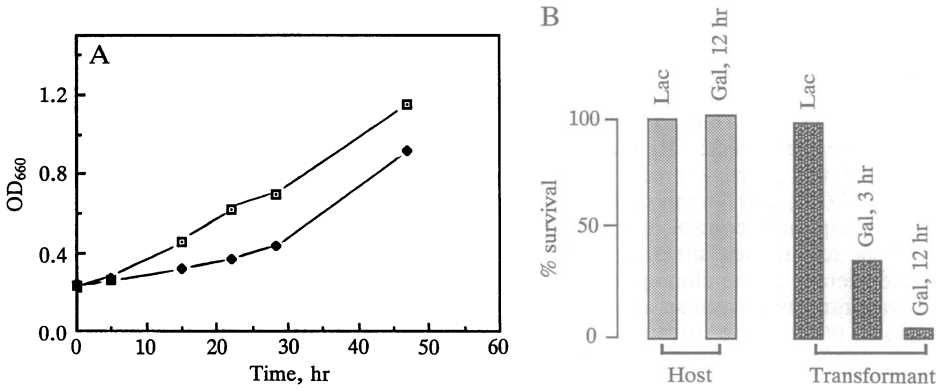

FIG. 2. (*A*) Growth curves. Galactose was added to cells grown on lactate to an OD_{660} of 0.25 and growth was recorded as a function of time. ⊡, Host; ◆, transformant. (*B*) Survival of cells was monitored by the colony-forming-unit test: a given number of cells were plated on 2% agar plates, containing YPD (18) and colonies were counted after 3 days.

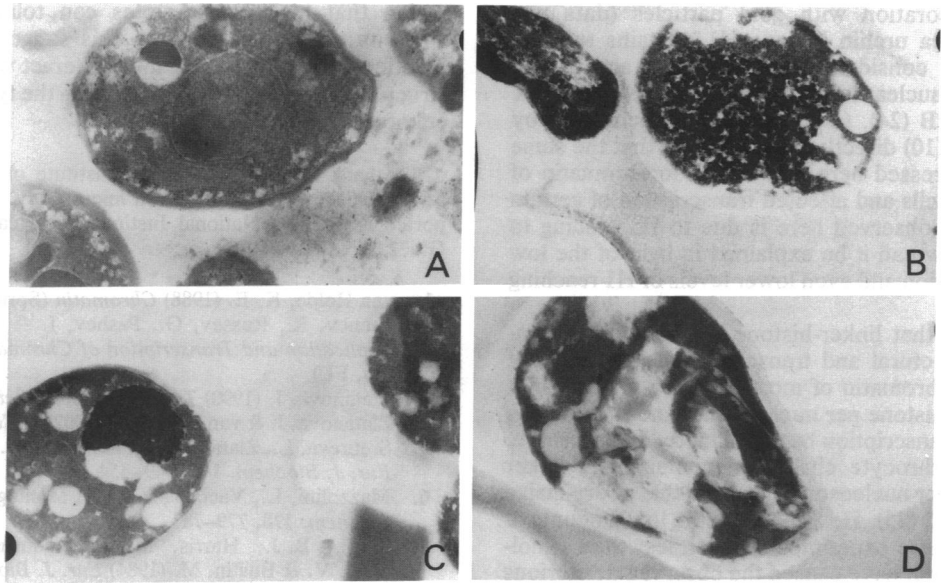

FIG. 3. Electron microscopic images of transformed cells cultivated on lactate (*A*) and on galactose-containing medium (12 hr after addition of galactose) (*B–D*). Note the dramatic changes in intracellular morphology upon H1 induction. (×14,800.)

sional denser nucleoli. In contrast, cultivation of the transformants, but not of the host cells, in galactose for 12 hr brought about dramatic morphological changes (Fig. 3 *B–D*). The central vacuole became extremely heterogeneous, with varying degrees of condensation of its contents, ranging from electron-transparent patches to areas of enormous density (see also Fig. 4). In some cases, the vacuolar membrane appeared severely damaged or was no longer discernable (Fig. 3*B*). Similar changes in the central vacuole have been reported in yeast cells grown under stress—during sporulation, for example—and have been interpreted as manifestations of the lysosomal functions of the vacuole (20). The damage of the vacuolar membrane probably occurs to liberate vacuolar hydrolytic enzymes (20). Other changes in galactose-induced transformed cells also occurred: the number of smaller vacuoles increased, mitochondria were no more clearly seen, and the cytosol appeared more granular. Only in a few cells could intact nuclei be detected, which explains our earlier unsuccessful attempts to isolate and study nuclei at this stage of H1 induction. Pictures taken at an earlier period of induction (3 hr after addition of galactose) revealed cells experiencing somewhat less obvious signs of cell damage (data not shown).

Localization of the Expressed H1 by Immunoelectron Microscopy. Finally, an attempt was made to localize the expressed H1 within cells by postembedding immunoelectron microscopy. Ultrathin sections of cells imbedded in Lowicryl (17) were reacted with specific anti-H1 antibodies, which were then visualized by reaction with gold particle-labeled second antibodies. At 3 hr of induction the number of gold particles was still only slightly above background, so all further experiments were performed on cells induced for 12 hr. The distribution of gold grains was more or less uniform throughout the cell, including the occasional seemingly intact nuclei. The cytoplasmic background was not surprising, since (*i*) the synthesis of the protein takes place in the cytoplasm and (*ii*) a cytoplasmic pool of histone H1 has been reported in higher eukaryotes (21, 22). Interestingly, an accumulation of grains was clearly observed in the cell walls and in the smaller vacuoles scattered throughout the cytoplasm. This localization probably reflects attempts to expel the non-self-protein from the cells.

From the immunolocalization data it seems that the majority of exogenous protein is dealt with even before reaching the nucleus. We believe, however, that some H1 does make it into the nucleus, for the following reasons. (*i*) Gold grains were observed in the few surviving nuclei. (*ii*) When the immunostaining was performed on nuclei isolated at 12 hr of induction by the method of Lohr (23), the very few structures recovered showed considerable chromatin condensation

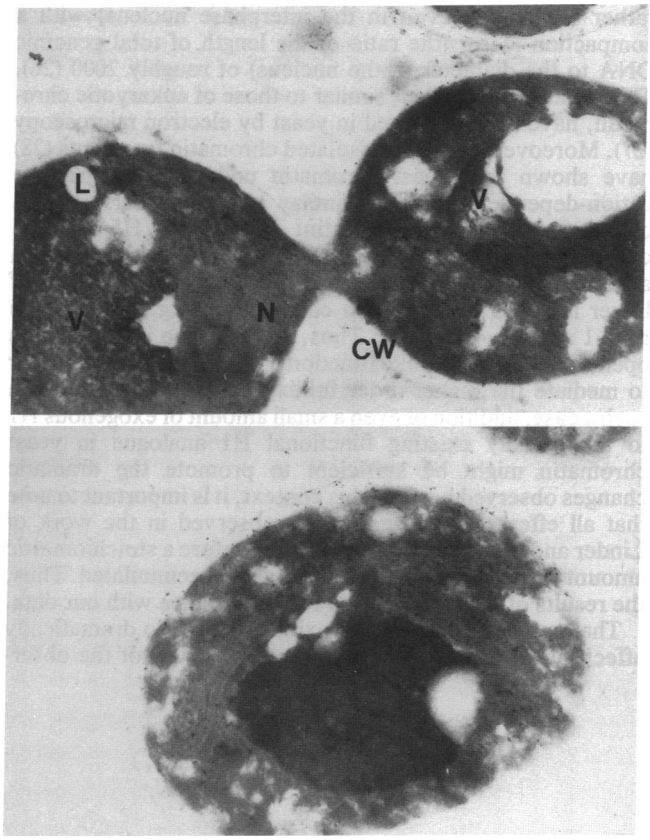

FIG. 4. Immunoelectron microscopic images of transformed cells cultivated in galactose-containing medium for 12 hr. N, nucleus; V, central vacuole; L, lipid globule; CW, cell wall. Note the uniform distribution of gold particles over the cells with some accumulation in the cell wall and smaller vesicles. (×14,800.)

with abundant decoration with gold particles (data not shown). (*iii*) The sea urchin histone H1 contains two sequence motifs with considerable homology (5 and 6 of 8 amino acids) to the nuclear localization signal KKRKSKAK in yeast histone H2B (24). (*iv*) The recent experiments by Linder and Thoma (10) directly demonstrated that the same protein as that expressed here was bound to chromatin of transformed yeast cells and affected transcription of certain genes. If the effect observed here is due to H1 binding to chromatin, then how can it be explained in light of the low levels of H1 expression and even lower levels of H1 reaching chromatin?

It is well known that linker-histone stoichiometry is important for the structural and transcriptional properties of chromatin (3, 4). Chromatin of most cells contains about 1 molecule of linker histone per nucleosome, and that of cells highly inactive in transcription has even more linker histone. Thus, chicken erythrocyte chromatin contains 1.3 linker histone molecules per nucleosome (18), and that of dry maize embryo cells about 2 (25). How then can the low amounts of exogenous H1 in these experiments, much less than 1 molecule per nucleosome, have caused the observed deleterious effect?

One possible explanation is that the majority of yeast genes seem to be essential for viability. In such a case, switching off transcription (by H1 binding) of any one critical gene could be detrimental to the cell. However, the effect on individual gene transcription is probably not the major cause of cell death. In the few nuclei still isolatable at 12 hr after H1 induction, chromatin was more highly condensed than in normal yeast nuclei. This hypercondensation may be achieved by the concerted action of the exogenous H1 and of an endogenous H1-like protein (5–7). Yeast DNA appears rather compacted even in the interphase nucleus, with a compaction factor (the ratio of the length of total genomic DNA to the diameter of the nucleus) of roughly 2000 (26). Thirty-nanometer fibers, similar to those of eukaryotic chromatin, have been observed in yeast by electron microscopy (27). Moreover, studies on isolated chromatin fragments (28) have shown that yeast chromatin undergoes progressive cation-dependent folding, similar to that observed with chicken erythrocyte chromatin; histone H1/H5-depleted chromatin from the same source was not able to condense, in accordance with numerous previous studies on the role of linker histones in chromatin compaction (e.g., ref. 29; see refs. 1 and 2 for reviews). Thus, although the issue remains open, a protein of H1-like function may exist in yeast in order to mediate the higher-order folding of yeast chromatin. In such a case, addition of even a small amount of exogenous H1 to the already existing functional H1 analogue in yeast chromatin might be sufficient to promote the dramatic changes observed here. In this context, it is important to note that all effects of exogenous H1 observed in the work of Linder and Thoma (10) occurred long before a stoichiometric amount of the protein was expressed and accumulated. Thus, the results of that study are in full accordance with our data.

That such low amounts of exogenous H1 so dramatically affect viability is especially intriguing in view of the observation that higher eukaryotes can tolerate much larger amounts of H1 (see above). This suggests that any yeast functional analogue of H1 must interact with the chromatin structure in ways quite different from the typical H1 of higher eukaryotes.

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