Nucleosome Structural Changes during Derepression of Silent Mating-type Loci in Yeast*

Thelma A. Chen-Cleland‡, M. Mitchell Smith§, Siyuan Le‖, Rolf Sterngranz‖, and Vincent G. Allfrey**

From the ‡Laboratory of Cell Biology, Rockefeller University, New York, New York 10021, the §Department of Microbiology, University of Virginia School of Medicine, Charlottesville, Virginia 22908, and the ‖Department of Biochemistry and Cell Biology, State University of New York, Stony Brook, New York 11794

Mutant a and a yeast cells were created with histone H3 containing cysteine in place of alanine 110. Because transcriptionally active nucleosomes “unfold” to reveal the histone H3-thiol groups at the center of the core, the active nucleosomes of the mutant strain can be isolated by mercury-affinity chromatography. We compared the unbound and mercury-bound nucleosomes of haploid H3-mutant strains expressing either the MATa or the MATa mating-type locus.

In a MATa strain, the Hg-bound nucleosomes are enriched in MATa DNA but lack the DNA of the transcriptionally silent HMRa mating-type locus. Conversely, in a MATa strain, the Hg-bound nucleosomes are enriched in MATa DNA sequences but deficient in HMLa DNA. When the SIR3 gene, known to be required for silencing of the repressed mating-type loci, is mutated in the MATa strain, transcription of the HMRa ensues, and its nucleosomes, as well as those of the MATa locus, are retained by the organomercurial column. It follows that derepression of the silent mating-type locus, caused by the sir-3 null mutation, is accompanied by an unfolding of its nucleosomes to reveal the histone H3 SH groups at their centers. Nucleosomes of the pheromone-encoding gene MFA2, a gene that is expressed in MATa cells but not in MATa cells, are bound to the organomercurial column when isolated from MATa cells but not from MATa cells. Thus, there is a good correlation between nucleosome unfolding and the renewed transcriptional activity at mating-type loci, and at MFA2, which had been silenced for prolonged periods. A close temporal correlation between nucleosome refolding and the cessation of transcription is not always observed in yeast, however, in contrast to observations in mammalian cells.

For example, nucleosomes of the GALI gene are maintained in a “poised” or “primed” thiol-reactive state even when the gene is not being transcribed (Chen, T. A., Smith, M. M., Le, S., Sterngranz, R., and Allfrey, V. G. (1991) J. Biol. Chem. 266, 6489–6498). It follows that the unfolding of the nucleosome cores of the yeast H3 mutant is regulated by factors that are not temporarily linked to the recruitment or traverse of the RNA polymerase complex, but which may determine the rate at which different domains of chromatin adapt to the need for transcription of the associated DNA sequences.

The fundamental repeating subunit of chromosome structure in yeasts, as in higher organisms, is the nucleosome. Its core contains 146 base pairs of DNA coiled in a helical ramp around a histone octet containing two molecules each of histones H2A, H2B, H3, and H4. In its central region, occupied by a histone (H3/H4)2 tetramer, two H3 molecules lie in close proximity across the dyad axis (reviewed in Ref. 1). Here we identify a fundamental change at the center of the nucleosome core when a silent mating-type gene of yeast is derepressed. That change permits the chromatographic isolation of nucleosomes containing transcriptionally active DNA sequences of the cell. The method is based on chemical and electron microscopic evidence that transcription of the ribosomal genes in Ph laserum is accompanied by an “unfolding” of the nucleosome core (2). In mammalian (3, 4) and avian (5) nucleosomes, as well as in Physarum (2, 6), this topographical change reveals the previously shielded thiol groups of histone H3 molecules located at the center of the core particle (7). Since the H3-thiols are not accessible to SH reagents in the compactly beaded nucleosomes of inactive genes (Refs. 2–4, and references cited therein), active and inactive nucleosomes can be separated by mercury-affinity chromatography (3, 4). The method has been shown to separate active from inactive nucleosomes of the c-fos, c-myc (8, 9), histone H4 (10), and ribosomal genes (11).

Although this procedure is effective in mammalian cells, which contain a cysteine residue at position 110 of the histone H3 polypeptide chain (12), it fails to bind the transcriptionally active nucleosomes of yeast (Saccharomyces cerevisiae) (4), an organism in which histone H3 has no cysteinyl-SH groups (13, 14). Therefore, to combine genetic experiments with the ability to separate and compare active and inactive yeast nucleosomes by Hg-affinity chromatography, we have prepared mutant yeast strains in which all the nucleosomes contain histone H3-thiols.

Site-directed mutagenesis was employed to replace the alanine codon (GCT) at position 110 of the yeast H3 amino acid sequence with a cysteine codon (TGT) (15). This corresponds to the cysteine 110 of mammalian histone H3 which binds active nucleosomes covalently to the organomercurial column (4). A plasmid containing the H3 Cys-110 allele, together with a normal histone H4 gene was used to construct a MATa yeast strain in which all the nucleosomes contain H3 molecules with cysteine in place of alanine-110 (15).
Nucleosome Unfolding at Derepressed Mating-type Loci

When nucleosomes of the H3-mutant strain were passed through the mercury column, about 24–30% bound to the column through their H3-thiol groups. The mercury-bound nucleosomes were found to be greatly enriched in the actively transcribed GAL1, HIS4, and ACT1 DNA sequences (15). Although this result confirms that nucleosomes along those genes occur in an unfolded, SH-reactive configuration, the situation is complicated by the fact that many yeast nucleosomes are maintained in a primed, potentially active state even when they are not being transcribed. For example, nucleosomes of the GAL1 gene bind to the mercury column when GAL1 expression is repressed during growth in glucose (15). Such persistence of unfolded, readily activated nucleosomes was not observed in mammalian genes such as c-fos and c-myc, in which nucleosomes reverted to the compact state within 10 min after RNA synthesis was inhibited (8, 9, 11); but it is probably the case for many loci in yeast, an organism which expresses most of its genome during log-phase growth (16, 17).

To investigate what happens to nucleosome structure along a yeast gene that is usually repressed but can be derepressed by mutation, we have focused on the sustained transcriptional silencing of the HMRα mating-type locus in MATα yeast cells (18, 19). There are three mating-type loci in yeast, located on chromosome III (20), all of which have been mapped and sequenced (21). The MAT locus, located near the middle of the right arm of the chromosome, is the expression locus that determines the mating type, which may be either MATα or MATα. In our H3-mutant strain, the active MATα locus is flanked by two unexpressed mating-type loci, HMLα and HMRα (located near the left and right ends of the chromosome), which serve as silent repositories of mating-type information. Transcription at the silent mating-type locus is repressed by trans-acting products of other genes, such as the SIR3 gene (22), which acts through cis-acting “silencer” elements located upstream and downstream of both HMLα and HMRα (23–25). Because mutation (26–31) of the SIR3 gene restores transcription at the HMRα locus, we decided to create a yeast strain containing both a sir3 null mutation and the gene for the thiol-containing histone H3. Mercuric affinity could then be used to test for unfolding of the nucleosomes at the derepressed HMRα locus.

Previous experiments had shown that the mercury-bound nucleosomes of a MATα strain expressing the mutant H3 Cys-110 gene contained the MATα mating-type DNA but lacked DNA of the silent HMRα locus (15). It was concluded that prolonged silencing of the HMRα gene is accompanied by compaction of its nucleosomes and loss of reactivity of their H3-thiol groups. Here we extend those studies to MATα yeast strains; and we examine what happens to nucleosome structure when the silent mating-type genes are derepressed in a MATα H3-mutant strain containing a sir3 null mutation.

EXPERIMENTAL PROCEDURES

Yeast Strains—The symbols HHT1 and HHF1 refer to the copy I histone H3 and H4 genes, while HHT2 and HHF2 refer to the copy II H3 and H4 genes (32). The haploid MATα strain MX17-18B expressing the histone H3 Cys-110 mutant allele hht2-2 has the genotype MATα, ura3-52, ade2-101, his3-Δ1, leu2-3-112, trpl-289, Δ(hht1-hh1f), Δ(hht2-hh2f), pMS339 (15). The haploid MATα strain MX17-2A has the genotype MATα, ura3-52, ade2-101, his3-Δ1, leu2-3-112, lys2-801, Δ(hht1-hh1f), Δ(hht2-hh2f), pMS339. The plasmid pMS339 contains a wild-type copy II histone H4 gene together with the mutant H3 gene (hht2-2) encoding the Cys-110 substitution. The strain R5912 has the genotype of strain MX17-18B plus the sir3Δ-TRP1 mutation (31). The wild-type yeast used was a prototrophic strain of S. cerevisiae BJ2168, and it has the genotype MATα, leu2, trp1, ura3-52, prbl-1122, pep4-5, prc1-407, gal2, constructed by E. Jones (Carnegie-Mellon University, Pittsburgh, PA).

Construction of Yeast Mutant Strains—The yeast histone H3 Cys-110 substitution mutant hht2-2 was prepared by oligonucleotide-directed mutagenesis, inserted into a shuttle vector (pMS339), and used to transform a yeast strain (MX17-18B) essentially dependent on the histone H3 Cys-110 allele, as described (15). The sir3Δ-TRP1 mutation was made by deleting a HpaI-EcoRI fragment from the SIR3 gene and inserting a 0.8-kb StuI-EcoRI TRP1 fragment to create plasmid pKL12. Plasmid pKL12 was cut with Sall and XhoI to yield the linear fragment used for gene replacement (31). The sir3Δ-TRP1 mutation was introduced into the strain MX17-18B expressing the H3 allele hht2-2 (15, 32) by one step gene replacement (33) to create strain R5912. The sir3Δ mutation was confirmed by Southern blot analysis and by the fact that the strain could not mate.

Isolation and Endonuclease Digestion of Yeast Nuclei—Yeast cells were grown at 29°C in YP-Glu (1% yeast extract, 2% peptone (Difco), 2% glucose), or YPGal (wherein galactose was substituted for glucose), until they reached a density of 1 × 106 cells/ml. Spheroplasts were prepared essentially as described by Jerome and Jaehning (34). The spheroplasts were lysed, and nuclei were isolated as described by Schulz (35).

The nuclei were washed in buffer A (10 mm Tris-HCl, pH 7.4, containing 25 mM KCl, 5 mM MgCl2, 0.35 mM sucrose, 5 mM sodium butyrate, 0.1 mM phenylmethylsulfonyl fluoride (Sigma), and 0.1 mM 1,2-epoxy-3-(paranitrophenoxy)propane (Kodak), and resuspended at a concentration of 20 μg of protein/ml. Incubation of 0.5 mM mercury (II) chloride and addition of 0.5 mM and addition of micococal nuclease (18 units/ml), the nuclei were incubated at 35°C for 5 min, at which time digestion was stopped by the addition of EGTA, pH 7.5, to a final concentration of 5 mm. The nuclei were centrifuged at 10,000 × g for 20 min, and the supernatant (S), representing 10.8 ± 2.6% of the total nuclear DNA (average of 21 experiments), was employed for chromatographic fractionation of nucleosomes.

Mercury-affinity Chromatography of Nucleosomes—The released nucleosomes were loaded on columns (1 × 6 cm) of Affi-Gel 501 (Bio-Rad) after washing with buffer B (10 mm Tris-HCl, pH 7.5, 25 mM KCl, 25 mM NaCl, 2% (w/v) sucrose, 5 mM sodium butyrate, 5 mM Na2EDTA, 0.1 mm phenylmethylsulfonyl fluoride, 0.1 mM 1,2-epoxy-3-(p-nitrophenoxy)propane) to remove unbound nucleosomes, and with buffer B containing 0.5 mM NaCl to displace nucleosomes adhering to the column through salt-labile associations with SH-containing nonhistone proteins, the nucleosomes covalently bound to the mercury column through the SH groups of the mutant histone H3e were eluted with 10 mM DTT in buffer B (15, 36).

DNA and Protein Determinations—The DNA contents of the chromatographically separated nucleosome fractions were determined by the bisbenzimide 33258 fluorescence method (37). The proteins of the nucleosome fractions were measured using the bicinchoninic acid assays (38), following dialysis to remove DTT. The proteins of the unbond and mercury-bound nucleosome fractions were subjected to electrophoresis in 18% polyacrylamide gels containing 0.1% SDS (39). The gels were stained with 0.25% Coomassie Brilliant Blue R-250, and the intensity of the stained bands was measured by laser-scanning densitometry.

DNA Sizing and Hybridization—DNA was prepared from the unbound and mercury-bound nucleosome fractions as described (3, 8, 40) and digested with 50 μg of RNase A for 1 h at 37°C, followed by 100 μg of proteinase K for 2 h at 37°C in the presence of 0.1% SDS. The DNA was extracted, precipitated in ethanol (40), and dissolved in 10 mm Tris-HCl, pH 7.4, 1 mm Na2EDTA for DNA sizing (41) and slot-blot hybridization experiments.

For DNA sizing experiments, 2 μg of each sample DNA was separated on 2% agarose gels in Tris-borate buffer as described (41). Agarose gels were stained with ethidium bromide and photographed under ultraviolet light as previously described (15, 41). The size and distribution of the DNA fragments of each sample was determined by scanning a negative of the gel with a laser densitometer and converting the molecular weight standard to kilobases.

In DNA hybridizations, 5 μg of DNA from each sample was blotted onto pretwisted nylon membranes (Zeta-Probe, Bio-Rad Laboratories) using a slot-blot apparatus (Schleicher and Schuell) and the alkaline procedure as described (15). The probe for the GAL1 locus was a 2-kb EcoRI fragment which also includes DNA sequences from the
transcribed region of the gene, excised from plasmid 4815 (15). The HIS4 DNA probe was the 1.2-kb SalI-BglII fragment from plasmid pFW48. The probe for ACT1 was the 1.6-kb HindIII-BamHI fragment from plasmid pCC68. GALI, HIS4, and ACT1 DNA probes were labeled with [32P]dCTP by random priming as described (40). The probes for the transcribed sequences of MATa and HMRa loci were prepared using plasmids pHS15 and pJH455, respectively, using [32P]UTP (300 Ci/mmol; Amersham). The MATa-specific pheromone gene, MPA2, a 1.8-kb DNA fragment which contains the MPA2 gene from plasmid pSM28, was labeled with [32P]dCTP by random priming. Prehybridization, hybridization, and washing of the membrane filters was carried out as described (8, 9, 15). After washing, the filter membranes were exposed to Fuji x-ray film for various times with a Du Pont Cronex I-G Plus intensifying screen at -80°C. After the x-ray films were developed in an Kodak X-Omat M4 developer, the autoradiograms were scanned with a laser densitometer (LKB Ultrascan 2920), and peak areas corresponding to each slot were integrated.

RESULTS AND DISCUSSION

Affinity Chromatography of Nucleosomes from Yeast Strains Expressing a Mutant Histone H3 Gene Encoding a Cys-110 Residue—Site-directed mutagenesis was used to create a histone H3 gene containing a cysteine codon in place, of the alanine codon for position 110 of the H3 amino acid sequence. This is the position of the cysteinyl residue in mammalian histone H3 (12) which is involved in the covalent binding of histone H3 of the 110 allele. A plasmid containing the H3 Cys-110 allele, together with a normal histone H4 gene, was used to construct haploid MATa and MATa yeast strains in which all of the nucleosomes contain H3 molecules with cysteine in place of alanine-110 (15).

Nuclei were isolated from the MATa and MATa H3-mutant strains and from wild-type MATa and MATa yeast strains. The nuclear preparations were treated with micrococcal nuclease to release 10.8 ± 2.5% of the total DNA (average of 21 experiments). More extensive digestions were avoided because the SH-reactive nucleosomes of transcriptionally active chromatin are degraded preferentially during more prolonged endonuclease treatments (2, 4).

In each case, the released nucleosomes were applied to an organomercurial-agarose column and, after washing the column to remove unbound nucleosomes (peak 1), the mercury-bound nucleosomes were eluted in two steps by a procedure that discriminates between two different modes of nucleosome binding (4). The first step uses 0.5 M NaCl to displace nucleosomes adhering to the mercury-column through salt-labile associations with SH-containing nonhistones proteins (peak 2). The second step uses 10 mM DTT to elute nucleosomes bound covalently to the phenyl-mercury groups of the column (peak 1). The released nucleosomes were applied to an organomercurial-agarose column and, after washing the column to remove unbound nucleosomes (peak 1), the mercury-bound nucleosomes were eluted in two steps by a procedure that discriminates between two different modes of nucleosome binding (4). The first step uses 0.5 M NaCl to displace nucleosomes adhering to the mercury-column through salt-labile associations with SH-containing nonhistones proteins (peak 2). The second step uses 10 mM DTT to elute nucleosomes bound covalently to the phenyl-mercury groups of the column (peak 1). A characteristic ladder of nucleosomal DNA lengths usually contains about 45% monomers, 25% dimers, 15% trimers, and 15% oligomers (Table I). The DNA length of the monomers is about 160 bp. All four core histones, H2A, H2B, H3, and H4, are present in stoichiometric proportions (Fig. 2B; Table II), confirming the presence of intact nucleosomes in the DTT-eluted fractions of the yeast strains expressing the H3-Cys-110 allele.

In contrast, the DTT-eluted fraction of wild-type yeast cells is small (Fig. 1A). Although it contains some high molecular weight DNA and many fragments smaller than mononucleosomal DNA, very few nucleosome-sized DNA fragments are detected (Fig. 2A), and there is a marked deficiency of histones relative to the DNA present (Fig. 2B) (15). This paucity of nucleosomes in the DTT-eluted fraction of wild-type yeast confirms that the introduction of the cysteine 110 residue in histone H3 of the mutant strains permits binding of their “unfolded” nucleosomes to the mercury column through the H3-thiol groups.

Contrasting Distribution of Mating-type DNAs in Nucleosome Fractions of Wild-type and H3-mutant Yeast Cells—In previous tests for specific DNA sequences in nucleosome fractions of the H3-Cys-110 mutant, the nucleosomes eluted in 10 mM DTT (peak 3) were enriched in the transcribed GALI, HIS4, and ACT1 genes; but a negligible recovery of those sequences appeared in the corresponding fraction of wild-type cells in which histone H3 has no cysteinyl residues (15). Although this is clear evidence that nucleosomes retained on the mercury column because of the presence of accessible H3-thiol groups include transcriptionally active DNA sequences, it does not constitute a proof that all the recovered nucleosomes were being transcribed at the time. In fact, the close temporal correlation between transcription and nucleosome “unfolding” as observed in mammalian cells (8, 9, 11) is not always observed in yeast cells. For example, 38% of the released GALI nucleosomes continue to bind to the mercury column when GALI transcription is repressed during culture in glucose (15). We conclude that many nucleosomes on the GALI gene remain poised in an unfolded state, “primed” for rapid transcription when galactose is added to the culture medium (42, 43). This persistence of an altered,
Nucleosome Unfolding at Derepressed Mating-type Loci

TABLE I
Proportions and DNA lengths of nucleosome fractions separated by mercury-affinity chromatography

| Yeast strain      | Nucleosome fraction | Distribution of DNA | Nucleosomes present asa | Monomeric DNA lengthsb |
|-------------------|---------------------|---------------------|-------------------------|------------------------|
|                   |                     | % of totalc         | % Monomers | Dimers | Trimmers | Oligomers | bp |
| Wild-type MATa    | Peak 1              | 72.6                | 45 | 23 | 12 | 20 | 162 |
|                   | Peak 2              | 21.3                | 33 | 26 | 17 | 24 | 186 |
|                   | Peak 3              | 7.1                 | Trace | Trace | Trace | 167 |
| H3-mutant MATaα   | Peak 1              | 52.6                | 46 | 36 | 15 | 3 | 160 |
|                   | Peak 2              | 17.7                | Trace | 46 | 25 | 29 | 160 |
|                   | Peak 3              | 29.7                | 43 | 29 | 20 | 8 | 160 |
| H3-mutant MATaα   | Peak 1              | 86.0                | 45 | 28 | 17 | 10 | 165 |
|                   | Peak 2              | 2.7                 | 42 | 21 | 7 | 173 |
|                   | Peak 3              | 11.3                | 44 | 25 | 15 | 16 | 167 |
| H3-mutant MATaα sir3 | Peak 1 | 43.2               | 39 | 30 | 18 | 13 | 165 |
|                   | Peak 2              | 18.2                | 25 | 42 | 26 | 7 | 190 |
|                   | Peak 3              | 38.6                | 38 | 25 | 12 | 25 | 167 |

a Proportion of peak DNA present as mono-, di-, tri-, and oligonucleosomes, as determined by laser-scanning densitometry of the ethidium-stained DNA bands in DNA-sizing gels.

b Size, as determined by the position of the absorption maximum of each DNA band relative to the migration distances of φX174 HaeIII fragments or 106-bp DNA ladder standards (Pharmacia).

c Proportion of total DNA applied to the Hg column recovered in each peak.

d DNA predominantly present as large pieces that did not enter sizing gel and fragments smaller than mononucleosomal DNA.

TABLE II
Histone composition of mercury-bound (peak 3) fractions from wild-type and H3-mutant yeast cells separated by mercury affinity chromatography

| DTT-eluted fractiona | Histone type | Histone proportion |
|----------------------|--------------|--------------------|
|                      | % totalb     |
| H3-mutant MATα       | H2A          | 24.3 ± 1.8          |
|                      | H2B          | 22.4 ± 2.6          |
|                      | H3           | 29.9 ± 2.0          |
|                      | H4           | 23.9 ± 5.0          |
| H3-mutant MATα       | H2A          | 25.7 ± 1.3          |
|                      | H2B          | 18.8 ± 2.4          |
|                      | H3           | 27.7 ± 2.1          |
|                      | H4           | 27.4 ± 5.4          |
| H3-mutant MATa sir3  | H2A          | 19.6 ± 2.7          |
|                      | H2B          | 25.9 ± 7.9          |
|                      | H3           | 30.7 ± 3.2          |
|                      | H4           | 23.8 ± 2.6          |
| Wild-type MATα       | H2A‘         | 5.3 ± 2.0           |
|                      | H2B‘         | 40.3 ± 1.6          |
|                      | H3‘          | 12.5 ± 3.1          |
|                      | H4‘          | 40.7 ± 1.5          |

a Peak 3.

b Histone proportion as determined by laser-scanning densitometry of Coomassie Blue R-250-stained SDS-polyacrylamide gels. Average of five determinations ± S.E.

c Protein bands migrating at approximate positions of respective histones.

is completely repressed for long periods? The complete and sustained transcriptional silencing of the HMRa mating-type locus in MATα yeast cells (28) was shown to result in a refolding of its nucleosomes to a non-thiol-reactive configuration, as evidenced by the very low recoveries of HMRa DNA (only 1.6–3.1%) in the mercury-bound nucleosomes (peak 3) of the MATα strain (15).

Here we extend the analysis of chromatin structure in active and inactive mating-type loci by analyzing the thiol reactivity of nucleosomes from the expressed and silent mating-type loci of a MATα yeast strain containing the H3-Cys-110 mutation. As before, the nuclei were isolated from the H3 mutant cells and digested with micrococcal nuclease to release 10 ± 3.4% of the total DNA. The released nucleosomes were applied to organomercurial columns for chromatographic separation

FIG. 2. DNA sizes and protein complements of the mercury-bound, DTT-eluted fractions (peak 3) of wild-type and H3 mutant yeast strains. A, the DNA size distribution in each fraction, as determined electrophoretically (41), is shown as follows: lane 1, DNA size standards; lanes 2 and 3, DTT-eluted fraction of wild-type yeast. Note the paucity of nucleosomal DNA lengths. Lane 4, DNA of DTT-eluted nucleosomes of the MATα H3 Cys-110 mutant yeast; lane 5, DNA of DTT-eluted nucleosomes of the MATα H3 Cys-110 mutant yeast; lane 6, DNA of DTT-eluted nucleosomes of the MATα sir3 hht2-2 double mutant yeast; lane 7, DNA size standards. B, electrophoretic analysis in SDS-polyacrylamide gels (39) of the proteins in the DTT-eluted fractions of wild-type and H3 mutant yeast strains. Lane 1, protein standards used as molecular weight markers; lane 2, DTT-eluted proteins of wild-type yeast; lane 3, proteins of DTT-eluted nucleosomes of MATα H3 Cys-110 mutant yeast; lane 4, proteins of DTT-eluted MATα H3 Cys-110 mutant yeast; lane 5 proteins of DTT-eluted nucleosomes of the MATα sir3 hht2-2 double mutant; lane 6, calf thymus core histones. Note the paucity of histones in the wild-type DTT eluate, and the prominent bands for all four core histones in the DTT eluates of the H3-mutant strains. See Table II for histone stoichiometry.

more accessible state of nucleosomes is probably the case for many yeast genes, as indicated by the earlier observation that there is little difference in the DNase I sensitivity of transcribed and bulk chromatin of yeast (17).

What happens to nucleosome structure when a yeast gene
of the unbound (peak 1), 0.5 M NaCl-eluted (peak 2), and DTT-eluted (peak 3) fractions (Fig. 1C). The DNA of each fraction was probed for its content of mating-type DNA sequences by slot-blot hybridizations to 32P-labeled riboprobes for the expressed MATa and the silent HMLα loci. (Because of the complete sequence homology throughout the transcribed regions of MATa and HMLα, and between MATα and HMLα (28), we used riboprobes for HMLα and MATα DNA to detect the cognate MATα and HMLα DNA sequences).

The slot blots (Fig. 3A) for the expressed MATα gene show enrichment of its sequences in the DTT-eluted nucleosomes of the MATα H3-mutant strain. As expected, the same probe detects the DNA of the silent HMLα locus in the unbound nucleosomes of peak 1. Hybridizations of the various nucleosomal DNA to the riboprobe for the silent HMLα locus show its sequences to be present in the unbound nucleosomes; but no appreciable hybridization signal was evident in the DTT-eluted nucleosome fraction (Fig. 3A). Control experiments, using wild-type yeast of the MATα mating-type, show that virtually none of the MATα sequences are recovered in the DTT-eluted fraction. Instead, those transcribed sequences appear in the unbound nucleosome fraction (Fig. 3A).

The results on the H3 mutant MATα yeast strain are to be contrasted with those obtained using a MATα H3-mutant strain (Fig. 3A), which show a strong hybridization signal for MATα DNA in the mercury-bound nucleosomes and a negligible signal for the transcriptionally silent HMRα DNA in that fraction (Fig. 3A). A control experiment using a wild-type MATα strain shows that the DTT-eluted fraction is depleted of the transcribed MATα DNA sequences (15). Thus, the experiments with strains of both mating types establish a clear correlation between nucleosome unfolding and transcription at the active MATα or MATα cassette; and they also confirm that nucleosomes at the transcriptionally silent HMRα and HMLα loci are maintained in a compact, non-thiol-reactive configuration.

We next investigated what happens to nucleosome structure when a silent mating-type locus is derepressed.

**Nucleosome Structural Changes during Derepression of the Silent HMRα Locus**—Transcription at the silent HM mating-type loci is repressed by the products of four unlinked genes known as the SIR (silent information regulator) genes (22, 43–44). Because mutation (26–31) of the SIR3 gene restores transcription at the HMRα locus, we decided to construct a yeast MATα strain containing both a sir3 null mutation and the gene for the thiol-containing histone H3. Mercury affinity was subsequently used to test for unfolding of the nucleosomes at the derepressed HMRα locus.

When the sir3 mutation was introduced into the H3 Cys-110 mutant strain, the MATα locus remained active, and the silent HMRα gene was derepressed, as indicated by Northern blot hybridizations (data not shown) and by the nonmating phenotype of the sir3 mutant cells. Separation of the active and inactive nucleosome fractions by mercury-affinity chromatography (Fig. 1D) revealed a characteristic ladder of nucleosomal length DNA fragments (Fig. 2A) and a full complement of core histones in the DTT-eluted fraction of the sir3 Cys-110 mutant (Fig. 2B, Table II). That fraction, which contains the thiol-reactive, unfolded nucleosomes of the double mutant, was analyzed for its content of HMRα and MATα DNA sequences by slot-blot hybridizations to the respective riboprobes. The results (Fig. 3A) show that both mating-type genes are present in the DTT-eluted nucleosomes in approximately equal amounts; 52.2% of the total MATα DNA and 47.3% of the total HMRα DNA applied to the column, as judged by densitometry of the slot blots (Fig. 4A).

It follows that derepression of the HMRα locus is accompanied by a change in nucleosome conformation that affects the accessibility of the histone H3-thiol groups located at the center of the nucleosome core.

To confirm that this is not the result of a widespread, nonspecific alteration of nucleosome structure due to the sir3 mutation, we investigated the chromatin structure of a pheromone-encoding gene, MFA2, which is known to be repressed in MATα cells, regardless of whether they contain a functional SIR3 gene. Little, if any, MFA2 DNA was recovered in the thiol-reactive nucleosomes of the MATα strain (Fig. 3B), indicating that no such global change had occurred throughout the chromatin of the sir3 mutant cells. We conclude that nucleosomes of the repressed MFA2 gene, like those of the silent HM mating-type loci, but unlike those of the repressed GAL1 gene (15; Fig. 4B) remain in a compactly beaded configuration. As expected, nucleosomes containing MFA2 bind to the column in MATα cells (Fig. 3).

**CONCLUSION**

The introduction of a cysteine residue in place of alanine 110 of yeast histone H3 has made it possible to detect the
“unfolding” of nucleosomal core particles by the change in reactivity of the centrally located H3-thiol groups. Because those SH groups are not accessible in the compactly beaded nucleosomes of transcriptionally inactive genes, the unfolded and compact nucleosomes of the H3-mutant yeast strains can be separated by mercury-affinity chromatography. This procedure fails to separate the unfolded nucleosomes of wild-type yeast cells in which histone H3 has no cysteinyl residues (4).

By comparing the DNA sequences of the mercury-bound nucleosomes with those of nucleosomes that do not bind to the mercury column, one can identify and recover the gene sequences that are being transcribed (or remain poised for activation) and those that are not being transcribed at that time. The distribution of transcribed and repressed mating-type DNAs provides a convincing demonstration of the separation procedure; hybridizations of the mercury-bound and unbound nucleosomal DNAs to riboprobes for MATa and HMRa, or for MATa and HMLa, confirm that nucleosomes in the transcribed domain of the expressed mating-type locus have unfolded; while nucleosomes in both of the silent HM loci remain in a compact, non-thiol-reactive configuration (Figs. 3A and 4A).

When the HMRa mating-type locus is derepressed as a consequence of a sir3 mutation, the nucleosomes along the gene unfold to reveal the hidden H3-thiols; the HMRa DNA appears, together with the DNA of the expressed MATa locus, in the mercury-bound nucleosome fraction. This unfolding of nucleosomes at the derepressed HMRa locus would be expected to increase their susceptibility to endonuclease attack, as observed, for example, in the transcribed nucleosomes of ribosomal chromatin (2, 11, 45). This is in accord with earlier observations by Nasmyth that SIR3 mutations “smear” the HMLa nucleosome profiles generated by micrococcal nuclease digestion (28). He concluded: “SIR regulation at the HM loci causes fundamental changes in chromatin structure and is clearly capable of action at a distance.”

Here we show that the change induced by the SIR3 mutation involves an unfolding of the nucleosome cores in the transcribed DNA sequences of the derepressed mating-type locus. How this control of nucleosome topography is achieved is not known, although considerable evidence now indicates that hyperacetylation of the core histones is a major contributory factor to an altered conformation at the center of the nucleosome core (3–6, 15, 46–51). For example, immunoprecipitation of yeast chromatin using antibodies to hyperacetylated histone H4 shows that the silent mating-type loci are underrepresented in the acetylated chromatin fraction when isolated from sir3 cells, but they are present at normal levels in the hyperacetylated chromatin from sir3 mutant cells (51).

That acetylation alters nucleosome topography to increase the accessibility of DNA at the mating-type loci is strongly supported by the observations showing that reactivation of the silent mating-type cassettes HMLa and HMRa greatly increases their modification by DNA-methylases (62), that the accessibility of HM loci to the HO-encoded nuclease increases when those loci are expressed (53), that UV-induced DNA damage at the active MATa locus is repaired preferentially to that at the inactive HMLa locus (54), and that this preference is not observed in a sir3 mutant where both loci are active (54).

The essential role of chromatin structure in the mechanism of mating-type repression is also evident in the observations showing that mutations of the conserved amino-terminal sequence of histone H4 (amino acids 16–19) specifically activate the silent HMLa locus (55–57); one of those residues (lysine 16) is critical for silencing, and its acetylation could lead to derepression of the silent mating loci. Recent work has shown that an adjacent sequence in histone H4 (amino acids 21–29) is also critical for silencing, and that all of the mutations in that region were suppressed in a sir3R background (58). This strongly suggests that the amino-terminal region of H4 may function as a recognition site for a specific repressor protein. The importance of chromatin structure in silencing of mating-type genes is also indicated by the finding that nucleosomes are stably and precisely positioned over the binding site for the o2 repressor protein (59).

Now, with the aid of yeast genetics, the problem can be approached in a new way, by the use of the H3-mutant strains we have described and the chromatographic techniques for the separation of active and inactive nucleosomes.

Acknowledgments—We thank Emmanuel Valentin for excellent and conscientious technical assistance and Stephen E. To for advice.
and assistance in computer-assisted plotting. We are indebted to Dr. James Haber of Brandeis University for the generous gift of the MATα and HMα probes, to Dr. Fred Winston of Harvard University for generously providing the GAL1, HIS3, and ACT1 probes, to Drs. John Arias and Michael Rout for providing advice and the wild-type yeast strains, and to Dr. S. Fields of the State University of New York, Stony Brook, for providing the MFA2 DNA probe.

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