DNA Sequence Plays a Major Role in Determining Nucleosome Positions in Yeast CUP1 Chromatin*

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Received for publication, May 23, 2001, and in revised form, July 17, 2001
Published, JBC Papers in Press, July 18, 2001, DOI 10.1074/jbc.M104733200

The role of DNA sequence in determining nucleosome positions in vivo was investigated by comparing the positions adopted by nucleosomes reconstituted on a yeast plasmid in vitro using purified core histones with those in native chromatin containing the same DNA, described previously. Nucleosomes were reconstituted on a 2.5 kilobase pair DNA sequence containing the yeast TRP1ARS1 plasmid with CUP1 as an insert (TAC-DNA). Multiple, alternative, overlapping nucleosome positions were mapped on TAC-DNA. For the 58 positioned nucleosomes identified, the relative positioning strengths and the stabilities to salt and temperature were determined. These positions were, with a few exceptions, identical to those observed in native, remodeled TAC chromatin containing an activated CUP1 gene. Only some of these positions are utilized in native, unremodeled chromatin. These observations suggest that DNA sequence is likely to play a very important role in positioning nucleosomes in vivo. We suggest that events occurring in yeast CUP1 chromatin determine which positions are occupied in vivo and when they are occupied.

Eukaryotic DNA is packaged into chromatin, the basic structural repeat unit of which is the nucleosome. The nucleosome core contains 147 bp1 of DNA wrapped in about 1.75 superhelical turns around a central octamer composed of two each of the four core histones H2A, H2B, H3, and H4. Its structure has been solved at high resolution by x-ray crystallography (1). In recent years, it has become clear that the nucleosome is not just a means of packaging large amounts of DNA into the nucleus but is also involved in regulation of gene expression (2, 3). The latter role stems from the fact that sequence-specific DNA-binding proteins often cannot recognize their sites when they are tightly wrapped in a nucleosome. The solution to this problem apparently resides in the activities of the many different chromatin remodeling and histone acetylase complexes recently identified. These facilitate the disruption or displacement of nucleosomes, allowing factors to bind, transcription complexes to form, and RNA polymerase II to initiate transcription.

One of the most interesting aspects of nucleosome structure is that the histone octamer does not bind to DNA randomly but exhibits preferences for some sequences over others, a phenomenon referred to as nucleosome positioning (4, 5). Positioning is actually determined by the central H3-H4 tetramer (6). The translational position of a nucleosome is defined by the 147-bp DNA sequence it occupies (see Ref. 7 for a detailed analysis). The affinity of the histone octamer for a particular 147-bp DNA sequence defines its positioning strength. Strongly positioned nucleosomes are those that contain sequences that bind the octamer much more tightly than neighboring sequences. There are many examples of these, the most well known being the 5 S RNA gene (8). There is a great deal of evidence for the importance of positioned nucleosomes in gene regulation (4). In budding yeast, the best studied example is that of the four nucleosomes positioned on the PHO5 promoter that are disrupted or displaced when the gene is activated for transcription (9).

Attempts to understand the mechanism of nucleosome disruption or displacement at various promoters led to the discovery of chromatin remodeling activities (10, 11). These are large, multisubunit complexes that use the energy of ATP hydrolysis to remodel chromatin structure in vitro in a variety of ways, including conformational changes in the nucleosome (12–14) and movement of nucleosomes between positions in vitro (15–19). We have shown recently that nucleosome repositioning also occurs in vivo and that this process requires the presence of the transcriptional activator (20).

We have adopted CUP1 as a model for understanding the role of chromatin structure in gene regulation. It encodes a metallothionein responsible for protecting cells from the toxic effects of excess copper ions (21, 22). Its regulation is relatively simple and well understood (23, 24): the N-terminal domain of a transcriptional activator, Ace1p (also called Cup2p), exhibits copper-dependent DNA binding at upstream activating sequences (UASs) in the CUP1 promoter. Ace1p activates transcription through its C-terminal acidic activation domain. The CUP1 promoter also contains two consensus TATA boxes (25) and an initiation element (26).

Recently, we described the native chromatin structure of a 2468-bp plasmid, TAC, purified from yeast cells, which contains CUP1 inserted into TRP1ARS1, an autonomously replicating yeast plasmid (20). Nucleosome positions were mapped over the entire TAC sequence using the monomer extension technique (27), revealing a complex chromatin structure with multiple, overlapping nucleosome positions (see Fig. 4A for a summary). In the presence of copper-activated Ace1p, 48 positioned nucleosomes were mapped in TAC minichromosomes. In its absence, the chromatin structure was simplified by the elimination of 16 of the positions on CUP1 and immediate flanking regions. The nucleosomes remaining on CUP1 were organized in clusters of positions separated by linker DNA of various lengths. It was proposed that Ace1p recruits a remodeling complex to the CUP1 promoter, which then directs the repositioning of nucleosomes over the entire gene and its...
flanking sequences.

Here, we have investigated the role of DNA sequence in determining nucleosome positions in vitro by comparing the positions adopted by nucleosomes reconstituted on TAC-DNA in vitro using purified core histones with those in native TAC chromatin. We demonstrate that the positions adopted by reconstituted nucleosomes in vitro are very similar to those observed in native, remodeled TAC minichromosomes. The nucleosomes positioned on inactive, unremodeled CUP1 represent a restricted subset of the same positions. Thus, DNA sequence plays a major role in determining nucleosome positions in yeast CUP1 chromatin.

MATERIALS AND METHODS

Nucleosome Reconstitution—The 261-bp SpeI-BsrGI CUP1 promoter fragment was obtained from pCP2 (20). The fragment was treated with phosphatase, purified from a gel, and end-labeled using T4 polynucleotide kinase. Reconstitution was as described (28), with modifications as follows. Labeled DNA fragment (4 μg) was mixed with chicken erythrocyte core histones at a ratio of 0.9 histone octamer per DNA fragment in a final volume of 300–400 μl of 2 mM NaCl, 10 mM HEPES-Na (pH 7.5), 1 mM Na-EDTA, 0.1 mM 4-2-aminoethylbenzenesulfonfonyl fluoride (AEBSF) (HEM1 buffer). For reconstitution of pGEM-TAC (20), 6 μg of purified core histones were mixed with either 10 or 40 μg of DNA (high and low input ratios, respectively) in a final volume of 400 μl of buffer as above. The mixture was dialyzed into the same buffer containing 5 mM urea overnight at 4 °C, using SpectraPor 7 dialysis tubing (molecular weight cut off = 8000). The next day, the NaCl concentration was reduced in steps by dialysis for 1 h each into 1.2, 1.0, 0.8, and 0.6 mM NaCl in the same buffer. The urea was removed by dialysis into 0.6 mM NaCl for about 3 h. Finally, the salt was removed by dialysis overnight into 10 mM HEPES-Na (pH 7.5), 1 mM Na-EDTA, 1 mM 2-mercaptoethanol, 0.1 mM AEBSF (HEM1 buffer). Reconstitutes were stored at 4 °C. Nucleosomes reconstituted on the CUP1 promoter fragment were analyzed in native polyacrylamide gels (29).

Preparation of Nucleosome Core DNA and Nucleosome Mapping—Reconstitutes were adjusted to 10 μg of DNA/ml in 40 mM NaCl, 2 mM CaCl2, 10 mM HEPES-Na (pH 7.5), 1 mM Na-EDTA, 1 mM 2-mercaptoethanol, 0.1 mM AEBSF and warmed briefly to 37 °C. Micrococcal nuclease (MNase) ( Worthington) was added to 0.1 unit/μg of DNA, and 300–400 μl aliquots were removed after 1, 2, 3, and 6 min at 37 °C. EDTA was added to 10 mM, SDS to 1%, and potassium acetate to 1M. DNA was extracted with chloroform and precipitated with isopropanol in the presence of 20 μg of glycogen. Core DNA was purified from a 6% (v/v) polyacrylamide gel (the time point with a tight core DNA size distribution was chosen; usually 3 min). For the experiments shown in Fig. 3, pGEM-TAC reconstituted at low histone:DNA ratio was concentrated using a Centricon-30 device (Millipore) prewashed with HEM1 buffer and digests with MNase as above. Monomer extension mapping of reconstituted and end-labeled nucleosome core DNA was digested with the restriction enzymes indicated. Control, no digestion. M, as defined in A. Each nucleosome gives rise to two bands totaling about 147 bp; the numbers refer to pairs of bands attributed to the nucleosomes in C. The lanes are all from the same gel but have been separated for clarity. C, map of nucleosome positions on the CUP1 promoter fragment. The initiation element (IE), the proximal and distal TATA boxes, and upstream activating sequences (UASs) are shown. The nucleosomes are numbered 1–10 and are shown with their coordinates relative to the BsrGI end. The approximate fraction of the total each nucleosome represents is given at the right and indicated by shading. The coordinates with respect to the TAC minichromosome are shown at either side of each nucleosome.

RESULTS AND DISCUSSION

Positioned Nucleosomes Reconstituted on a DNA Fragment Containing the CUP1 Promoter—Nucleosomes were reconstituted by salt/urea dialysis (28) on an end-labeled 261-bp BsrGI-SpeI DNA fragment containing the CUP1 promoter, including both TATA boxes and the upstream activating sequences, using purified chicken erythrocyte core histones. Chicken histones were used rather than the ideal choice of yeast histones because of technical difficulties we encountered in preparing intact yeast histones. The products of reconstitution were analyzed in a native gel that preserves nucleosomal structure (Fig. 1A). Nucleosomes migrate in these gels according to their position with respect to the center of the fragment; nucleosomes positioned at one end of a fragment migrate faster than those at the center (29, 30). Thus, the major, fast migrating band represents nucleosomes positioned close to the end of the DNA fragment, and the slower, less intense band represents nucleosomes in more central positions. The most slowly migrating band is probably a histone-DNA aggregate.

To determine their precise positions, reconstituted nucleosomes were completely digested to core particles using MNase. Core particle DNA (∼147 bp) was extracted, purified from a gel and end-labeled with T4 polynucleotide kinase. The positions were determined by restriction mapping (29, 31) (Fig. 1B). When core DNA is digested with a unique restriction enzyme, two end-labeled fragments adding up to about 147 bp are obtained. A double digest is performed to determine which of the two fragments represents DNA upstream and downstream
of the restriction site, using a second enzyme that cuts only one of the two fragments.

Undigested core particle DNA (Fig. 1B, lane 1) migrated as a tight band adjacent to the 147-bp band in the marker, as expected. To determine the positions of nucleosomes located near the BsrGI end of the fragment, core DNA was digested with MfeI (Fig. 1B, lane 2), MseI (lane 3), or both enzymes (lane 4). Two main bands of 132 and 14 bp were obtained with MseI, adding up to 146 bp. The MseI site is only 15 bp from the BsrGI end of the DNA, and so the 132-bp fragment must derive from the other end; this nucleosome is positioned right at the end of the DNA, and so the 132-bp fragment must derive from nucleosome 1). Digestion of core DNA with MfeI gave rise to many bands, the most intense of which were 52–57, 62–66, 72, 76, 84, and 92–97 bp. These can be paired as follows to give totals of about 147 bp: 72 with 76 (totaling 148 bp), 84 with 62–66 (146–150 bp), and 92–97 with 52–57 (144–154 bp). Clusters of bands (e.g., 52–57 bp) were assumed to reflect different degrees of trimming of the same core particle by MNase. The first pair of bands corresponds to nucleosome 1; the 76-bp band is sensitive to MseI (Fig. 1B, lane 4) and so maps to the BsrGI end of the fragment. The second pair of bands (84 and 66) derives from a nucleosome at 11–157 (nucleosome 2; the 84-bp fragment must derive from the SpeI side of the MfeI site because there are only 73 bp on the other side). The third pair of bands (97 and 52–57 bp) defines nucleosome 3 at 21–170. The MfeI digest also gave rise to some weak bands at 21, 26, 34, 42–47, 105, 112, 121, and 128 bp, corresponding to nucleosomes 4–7 (Fig. 1C).

To determine the positions of nucleosomes located near the SpeI end of the fragment, core DNA was digested with XbaI (Fig. 1B, lane 5), AlwNI (lane 6), or both enzymes (lane 7). The major bands in the XbaI digest (lane 5) were 85 and 63 bp (totaling 148 bp). Because the XbaI site is only 66 bp from the SpeI end and the 63-bp band is sensitive to AlwNI (lane 7), this nucleosome must be positioned at 110–258 (Fig. 1C, nucleosome 10). The major bands (20–23 and 118 bp) in the AlwNI digest (Fig. 1B, lane 6) also correspond to this nucleosome. Nucleosome 9 at 99–224 is defined by the 96- and 47–54-bp bands (147–150 bp) in Fig. 1B, lane 5, and the 130- and 15-bp bands in lane 6. Nucleosome 8 at 77–221 is defined by the 118- and 31-bp bands (144 bp) in lane 5.

Quantitative analysis using a phosphorimager indicated that nucleosome 10 is the most dominant position, representing about 30% of nucleosomes. Nucleosomes 1, 2, 3, 8, and 9 accounted for 10–20% each. Nucleosomes 4–7 are relatively weak (only about 2% each). Nucleosomes 1, 2, and 3 might be rotationally related, because their positions differ by units of about 10 bp, the helical repeat of DNA. Nucleosomes 8, 9, and 10 might also be rotationally related. In conclusion, this CUP1 promoter fragment contains six strong and four weak nucleosome positioning signals.

The nucleosome positions mapped on the CUP1 promoter fragment were compared with those mapped previously on a plasmid containing CUP1 purified as native chromatin from yeast cells (20). This 2468-bp minichromosome TRPIARS1CUP1 (TAC) contained CUP1 inserted into the autonomously replicating yeast TRPIARS1 plasmid. There were four positioned nucleosomes present on the SpeI-BsrGI region in native TAC, which were numbered 25–28. Taking into account the standard errors reported by Shen et al. (20) (which reflect the difficulty of obtaining completely trimmed core particles from native chromatin), nucleosome 25 corresponds to reconstituted nucleosomes 1, 2, and perhaps 3. Nucleosome 26 in TAC corresponds to reconstituted nucleosomes 6 and 7. Nucleosomes 27 and 28 in TAC correspond to reconstituted nucleosomes 9 and 10, respectively. Thus, reconstituted nucleosomes adopted very similar positions to nucleosomes in native chromatin containing the CUP1 promoter. The next step was to determine to what extent this was true for the entire TAC sequence.

Mapping of Nucleosome Positions on Reconstituted TAC DNA by Monomer Extension—The positions adopted by nucleosomes reconstituted on the entire TAC sequence were mapped using the monomer extension method, which is ideal for long range mapping and can resolve arrays of overlapping positions in complex chromatin structures, without ambiguity (20, 27, 32, 33).

Nucleosomes were reconstituted on pGEM-TAC, a plasmid that contains the 2468-bp TAC sequence inserted using the HindIII site inside the TRP1 open reading frame (20). A map of TAC is shown in Fig. 2B; coordinates (1–2468) are numbered from the HindIII site, in the direction of TRP1 transcription. The reconstitute was digested completely with MNase to obtain core particles. Core DNA (147–160 bp) was purified from a gel and end-labeled with T4 polynucleotide kinase. Labeled core DNA was used as primer for extension by Klenow enzyme with single-stranded pGEM-TAC DNA as template. The replicated DNA was digested with different restriction enzymes (with unique sites) to resolve different regions of TAC. The lengths of the resulting DNA fragments were determined accurately in sequencing gels: each band defines the distance from the border of a nucleosome to the chosen restriction site. A control for sequence-dependent termination by Klenow enzyme involves omission of the restriction enzyme. The borders of each nucleosome were located precisely, one using the + strand as template, the other using the − strand. The data from one template strand define all of the “upstream” nucleosome borders unambiguously and are sufficient to define the chromatin structure. The other strand should give the same nucleosome positions, this time defined by the “downstream” borders. The degree to which the two sets of positioning data are consistent can be assessed by calculating the average distance between the nucleosome borders, which should be close to 147 bp, the size of the core particle. A slight underdigestion of chromatin by MNase results in core particles that are not completely trimmed to 147 bp. Consequently, bands close together are likely to represent different degrees of trimming of the same positioned nucleosome. In the analysis, clusters of bands within 10 bp were assumed to derive from the same nucleosome. If core particles are overdigested by MNase, nicking begins to appear. Labeled core DNA was routinely checked in denaturing gels; the size range was typically 140–160 bp, with little nicking. In any case, nicking would not affect the result, because kinase does not label nicks, and end-labeled nicked DNA strands liberated on denaturation of core DNA would give the correct result on extension.

Nucleosomes were reconstituted at high and low histone: DNA ratios, corresponding to 1 histone octamer per 260 or 1000 bp, respectively. Reconstitution at low ratios is expected to limit the influence of possible nucleosome-nucleosome interactions on positioning (27). Monomer extension mapping for the entire TAC insert is presented in Fig. 2 and data from several experiments are summarized in Table I. A total of 58 positioned nucleosomes were identified on the TAC sequence, many of which are overlapping and therefore mutually exclusive. The average distance between matched borders (Table I) was 148 ± 8 bp, as expected for core particle DNA. This indicates that the chromatin structures mapped in the analysis of each strand are in agreement. The band intensities (Fig. 2) were normalized to the strongest positions in the list, which were assigned a value of 10. Strongly positioned nucleosomes 1 and 2 (Table 1) both include the ABF1 binding site (coordinates 104–160), a naturally bent DNA in ARS1 (34); bent DNA typically positions nucleosomes strongly. There was a 10-fold range in positioning...
Fig. 2. Mapping of nucleosome positions on a plasmid containing the entire TRP1ARS1CUP1 (TAC) sequence using the monomer extension method. A, nucleosome core particles were prepared from pGEM-TAC reconstituted at low or high histone:DNA ratios (1 octamer per 1000 or 260 bp, respectively). Labeled core DNA was used for extension with the + strand of pGEM-TAC in the presence of the restriction enzymes indicated. These enzymes have unique sites in pGEM-TAC at coordinates 1269 (XcmI), 1833 (BamHI) and just inside the vector (SapI). Control lanes, extension but no digestion with restriction enzyme. The products were analyzed in a sequencing gel. The lanes are all from the same gel but have been separated for clarity. Nucleosome positions are defined by their + strand borders given as TAC coordinates and listed in Table I. They are indicated by dots or bars (the latter indicating bands that were included within that nucleosome position, as discussed in the text). Markers are HinfI and Ddel digests of α-DNA labeled with T4 kinase. B, map of nucleosomes formed on pGEM-TAC. TAC is shown as a 2468-bp insert at the HindIII site of the vector, with map coordinates indicated. The HindIII site is near the 3′ end of TRP1. GAL refers to a UAS that binds Gal4p belonging to the GAL3 gene just downstream of ARS1 in the yeast genome. CUP1 was originally inserted into TRP1ARS1 at the EcoRI site at coordinates 839–1854, as shown. Also shown are the TATA boxes and UASs belonging to CUP1; just upstream is the 3′ end of the gene neighboring CUP1 in the genome, referred to as URF. Underneath the map, nucleosome positions are indicated with their + strand borders (determined in A). The strongest positions (values of 5 or more; Table I) are drawn with thick lines. Below these are drawn medium-strength positions (values of 3–5) and weak positions (values of 2 or less; thinnest lines). Note that the nucleosomes can be arranged in many possible arrays.

Nucleosome Positions in Reconstituted TAC Chromatin Are Very Similar to Those Mapped in Native TAC Chromatin—In Table I, the nucleosome positions in the pGEM-TAC reconstituted are compared directly with those reported for native TAC chromatin purified from copper-induced cells (20). There is close agreement. Native chromatin contained 48 positions (one of these, nucleosome 48, includes TAC sequences on both sides of the HindIII site that was used to insert TAC into the vector and so cannot be formed on pGEM-TAC). The larger number of positions (58) in the reconstitute mostly reflects the smaller error in the current measurements. With the relatively small amounts of native TAC chromatin that it was possible to isolate, it was harder to obtain closely trimmed core particles, and consequently the error due to trimming was higher. Of the 47 native positions possible on pGEM-TAC, 35 (74%) are represented by nucleosome borders within 10 bp of those in vitro, and 41 (87%) are represented by a nucleosome border within 25 bp. The six positioned nucleosomes observed in native chromatin but not listed for reconstituted chromatin were actually detectable as very weak bands, but they were too weak to be included in Table I. It is concluded that a large fraction of the nucleosome positions observed in native chromatin are present...
### Table I

Comparison of nucleosome positions in reconstituted and native TAC chromatin

The table includes data from multiple monomer extension mapping experiments using both + and − strands and different restriction enzymes, as in Fig. 2. Nucleosome positions are numbered according to the TAC sequence, coordinates 1–2468, beginning at the *HindIII* site in *TRP1*, in the direction of *TRP1* transcription. In the cases where only one border coordinate is given, there was a small gap in the map for one strand. For comparison, data from Table 1 in Shen et al. (20) include native TAC chromatin, with the position number and coordinates of the nucleosomes mapped. Positioning strengths reflect quantitative analysis of gels such as that in Fig. 2, with the strongest band arbitrarily set at a value of 10. Strong effects of salt and temperature on positioning strength are included (data in Fig. 3).

| Nucleosome position | TAC coordinates in vitro | TAC coordinates (native chromatin) | Positioning strength at 4 °C, 40 mM salt | Effects of salt and temperature |
|---------------------|--------------------------|-----------------------------------|------------------------------------------|-------------------------------|
| 1.                  | 24–160                   | 1. 14–164                         | 10                                       |                               |
| 2.                  | 123–285                  | 3. 131–277                        | 9                                        | Enhanced at 0.6 M, 37 °C     |
| 3.                  | 209–345                  | 5. 197–352                        | 1                                        |                               |
| 4.                  | 247–398                  | 6. 244–390                        | 2                                        |                               |
| 5.                  | 274–414                  | 7. 292–453                        | 1                                        |                               |
| 6.                  | 339–470                  | 8. 353–452                        | 1                                        |                               |
| 7.                  | 359–535                  | 8. 410–545                        | 2                                        | Lost at 37 °C                |
| 8.                  | 414–563                  | 8. 410–545                        | 3                                        | Enhanced at 0.6 M, 37 °C     |
| 9.                  | 429–586                  | 9. 441–591                        | 2                                        | Lost at 37 °C                |
| 10.                 | 490–630                  | 10. 487–642                       | 3                                        |                               |
| 11.                 | 523–669                  |                                   | 6                                        |                               |
| 12.                 | 579–722                  |                                   | 8                                        |                               |
| 13.                 | 670–821                  | 12. 666–818                       | 9                                        | Lost at 37 °C                |
| 14.                 | 733–877                  | 13. 739–889                       | 7                                        |                               |
| 15.                 | 779–927                  | 14. 792–924                       | 6                                        |                               |
| 16.                 | 827–975                  | 15. 840–995                       | 1                                        |                               |
| 17.                 | 879–1030                 | 16. 872–1028                      | 4                                        |                               |
| 18.                 | 947–1103                 | 17. 923–1073                      | 3                                        | Appears at 0.6 M, 37 °C      |
| 19.                 | 963–1113                 | 18. 964–1120                      | 3                                        |                               |
| 20.                 | 993–1148                 |                                   | 4                                        |                               |
| 21.                 | 1048–1195                | 19. 1028–1188                     | 2                                        |                               |
| 22.                 | 1098–1240                |                                   | 2                                        | Lost at 37 °C                |
| 23.                 | 1133–1273                | 20. 1143–1291                     | 10                                       |                               |
| 24.                 | 1153–1308                | 21. 1143–1291                     | 10                                       |                               |
| 25.                 | 1170–1327                | 22. 1196–1347                     | 3                                        |                               |
| 26.                 | 1200–1343                | 23. 1238–1394                     | 3                                        |                               |
| 27.                 | 1235–1387                | 24. 1269–1429                     | 2                                        |                               |
| 28.                 | 1291–1438                | 25. 1311–1464                     | 5                                        |                               |
| 29.                 | 1314–1473                |                                   | 8                                        |                               |
| 30.                 | 1341–1487                |                                   | 3                                        | Enhanced at 0.6 M, 37 °C     |
| 31.                 | 1385–1511                | 26. 1367–1516                     | 3                                        |                               |
| 32.                 | 1389–1546                |                                   | 3                                        |                               |
| 33.                 | 1432–1569                | 27. 1413–1552                     | 2                                        | Reduced at 0.6 M on ice      |
| 34.                 | 1458–1597                | 28. 1475–1651                     | 1                                        |                               |
| 35.                 | 1469–1605                |                                   | 7                                        |                               |
| 36.                 | 1483–1653                |                                   | 1                                        | Enhanced at 0.6 M, 37 °C     |
| 37.                 | 1502–1649                |                                   | 2                                        | Enhanced at 0.6 M, 37 °C     |
| 38.                 | 1516–1669                |                                   | 2                                        |                               |
| 39.                 | 1558–1703                | 29. 1550–1690                     | 2                                        |                               |
| 40.                 | 1670–1824                | 30. 1651–1810                     | 1                                        |                               |
| 41.                 | 1704–1849                | 31. 1695–1866                     | 3                                        |                               |
| 42.                 | 1747–1893                | 32. 1749–1893                     | 1                                        |                               |
| 43.                 | 1795–1950                | 33. 1789–1939                     | 2                                        |                               |
| 44.                 | 1809–1974                |                                   | 2                                        |                               |
| 45.                 | 1837–1994                | 34. 1842–2005                     | 2                                        |                               |
| 46.                 | 1864–2015                | 35. 1842–2005                     | 2                                        |                               |
| 47.                 | 1919–2056                |                                   | 4                                        |                               |
| 48.                 | 1962–2100                | 36. 1973–2106                     | 3                                        |                               |
| 49.                 | 1974–2119                | 37. 1973–2106                     | 3                                        |                               |
| 50.                 | 1999–2156                |                                   | 1                                        |                               |
| 51.                 | 2034–2179                | 38. 2035–2179                     | 2                                        |                               |
| 52.                 | 2071–2214                | 39. 2071–2217                     | 4                                        |                               |
| 53.                 | 2126–2289                | 40. 2123–2286                     | 2                                        |                               |
| 54.                 | 2164–2311                | 41. 2163–2311                     | 3                                        |                               |
| 55.                 | 2177–2346                | 42. 2185–2346                     | 2                                        | Reduced at 0.15, 0.6 M, 37 °C|
| 56.                 | 2217–2369                | 43. 2221–2373                     | 3                                        |                               |
| 57.                 | 2247–2395                | 44. 2247–2411                     | 2                                        | Reduced at 0.15, 0.6 M, 37 °C|
| 58.                 | 2269–2419                | 45. 2270–2419                     | 1                                        |                               |

This is most obvious when the reconstituted chromatin is compared with native unremodeled chromatin; many positions specified by DNA sequence are not utilized in TAC chromatin purified from *ace1Δ* cells (20). Thus, which positions are occupied in *vivo* and when they are occupied is determined by events occurring in TAC chromatin.

**A Major Role for DNA Sequence in Determining Nucleosome Positions in Vivo**—It has long been appreciated that DNA
sequence has an important role in determining nucleosome positions \textit{in vivo} (35). Pioneering studies on nucleosome positioning revealed that the 5 S rRNA gene from various organisms contains a strong nucleosome positioning signal (8, 36) and that it is also recognized in yeast cells (37). It has since become clear that the nucleosome adopts one of several alternative overlapping positions on 5 S DNA \textit{in vitro} (30, 38, 39) and \textit{in vivo} (40). DNA sequence also plays a decisive role in the positioning of nucleosomes on mouse satellite DNA \textit{in vivo} (41, 42). Other examples include positioned nucleosomes on the mouse mammary tumor virus long terminal repeat (43–46) and the chicken $\beta$-globin gene (47).

An important factor in determining the occupancies of particular nucleosome positions is the presence or absence of transcription factors at their binding sites: examples for which evidence \textit{in vivo} is available include the binding of the winged helix transcription factor HNF3 at the mouse serum albumin gene enhancer (48, 49) and the glucocorticoid receptor at the mouse mammary tumor virus promoter (50). The yeast $\text{GAL1-10 UAS}$ region is an interesting case because it is nucleosome-free \textit{in vivo} but forms nucleosomes \textit{in vitro} (51). This might be an example of a DNA sequence that does not position nucleosomes \textit{in vivo}, or alternatively, it might reflect the presence of bound transcription factors that block nucleosome formation \textit{in vivo}.

\textit{Nucleosome Stability and Mobility in Reconstituted TAC Chromatin}—It has been shown by several groups that some positioned nucleosomes exhibit temperature-dependent mobility on DNA \textit{in vitro}, moving between positions, whereas others are stable (30, 52, 53). The stability of nucleosomes on TAC-DNA was investigated by incubating reconstituted TAC chromatin at salt concentrations and temperatures that promote mobility (low ionic strength at 37 °C in the absence of divalent cations) or sliding (high salt concentrations at 37 °C (54)). Preformed reconstitutes were incubated at salt concentrations of 40, 150 (approximately physiological), or 600 mM NaCl on ice or at 37 °C and then diluted to 40 mM NaCl in the presence of calcium ions to suppress mobility and for MNase digestion to core particles. The monomer extension analysis is shown in Fig. 3 and summarized in Table I.

Most nucleosomes were unaffected by changes in temperature and/or salt concentration. Increasing the salt concentration on ice affected only nucleosome 33 (with $\beta$ border at 1432; see Fig. 3A, BamHI), which was reduced in stability. Increasing the temperature to 37 °C resulted in the loss of positions 7, 9, and 13 (with $\beta$ borders at 389, 429, and 670) in the region neighboring $\text{ARS1}$ (in the XcmI panel of Fig. 3A) and positions 22 (1098) and 24 (1153) at the 3’ end of $\text{CUP1}$ (Fig. 3A, BamHI). The loss of the nucleosome at 670 (Fig. 3A, XcmI), which covers the UAS$_{\text{GAL}}$, is particularly striking, as it is a strong position at low temperature.

The combined effects of 600 mM salt and 37 °C were quite...
Nucleosome Positioning on Yeast DNA

In conclusion, the stabilities of most TAC nucleosomes are unaffected by incubation at physiological temperature and salt concentration (0.1–0.15 M). However, some are significantly destabilized at 37 °C. Under nucleosome sliding conditions (0.6 M NaCl, 37 °C), differently positioned nucleosomes are affected in opposite ways, with some stabilized and others destabilized. Of the 58 positions listed in Table I, 15 are sensitive to changes in salt concentration and temperature and 8 are affected at physiological salt concentration. The changes in nucleosome stability presumably reflect changes in the affinity of the histone octamer for particular DNA sequences, the conformations of which are sensitive to changes in salt and temperature. An important factor here might be the presence or absence of runs of dA or dT (55); competition experiments involving reconstitution on synthetic poly(dA)-poly(dT) have shown that nucleosome formation is relatively unfavorable at 4 °C, but relatively favored at 37 °C (56). However, most nucleosomes formed on TAC-DNA are stable at physiological salt concentration and temperature, implying that energy would have to be expended to move them from one position to another (see below).

Nucleosome Positioning and Stability on TAC-DNA—In summary, we have mapped the positions of nucleosomes reconstructed on the 2468-bp TRP1ARS1CUP1 sequence using purified core histones. The relative band intensities in the monomer extension maps indicate the relative occupancies of the various positions and reflect the relative affinities of the histone octamer for these DNA sequences under these conditions. There is an ~10-fold range in affinity from the weak positions to the strongest. This translates into modest differences in nucleosome stability, but the range does not include the many other possible 147-bp sequences in TAC that have no detectable positioning strength. Thus, transfer of a histone octamer from the strongest position to a weak position would cost much less energy than if it were transferred to a sequence with undetectable positioning strength.

Implications for the Remodeling of CUP1 Chromatin—In its inactive state, CUP1 is organized into clusters of alternative positioned nucleosomes separated by linkers of various lengths (Fig. 4A), which is likely to represent undisturbed chromatin assembled during DNA replication (20). In the presence of copper ions, Ace1p binds to its sites in the CUP1 promoter and directs the repositioning of nucleosomes from the clusters to the linkers, presumably by recruiting a remodeling activity. Reconstituted chromatin resembles the activated, remodeled state of native TAC, with all the positions represented, including those on the linkers.

The energy required to move a nucleosome from one position to another will be the sum of the difference in energy of nucleosome formation between the two positions (which is related to their relative positioning strengths) (Table I) and the activation energy required to move the nucleosome, which reflects the breakage and re-formation of all histone-DNA contacts (Fig. 4B). It is envisaged that the remodeling activity recruited...
by Ace1p uses the energy of ATP hydrolysis to overcome the energy barrier for movement. This activity would tend to distribute nucleosomes among the positions according to their relative positioning strengths, i.e., it would move the system toward equilibrium. (An example of a remodeling complex with this type of activity might be NURF (15).) Thus, the inactive gene represents a higher energy chromatin state that is destabilized only in the presence of remodeling activity. Using this argument, reconstituted chromatin would be expected to resemble remodeled rather than inactive chromatin.

Acknowledgments—We thank Gary Felsenfeld for helpful discussion and Ray Akhavan, Ann Dean, Jurrien Dean, Rohinton Kamakaka, Yeon-Jung Kim, and Benoit Leblanc for comments on the manuscript.

REFERENCES

1. Luger, K., Maeder, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) Nature 389, 251–260
2. Grunstein, M. (1997) Nature 389, 349–352
3. Wolffe, A. P. (1999) Chromatin, Ed. 3, Academic Press, London
4. Simpson, R. T. (1991) Proc. Nucleic Acids Res. Mol. Biol. 40, 143–184
5. Wallrath, L. L., Lu, Q., Grunz, H., and Elgin, S. C. R. (1994) Bioessays 16, 165–170
6. Dong, F., and van Holde, K. E. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 10596–10600
7. Lowary, P. T., and Widom, J. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1183–1188
8. Simpson, R. T., and Stafford, D. W. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 51–55
9. Svaren, J., and Horz, W. (1997) Trends Biochem. Sci. 22, 93–97
10. Kingston, R. E., and Narlikar, G. J. (1999) Genes Dev. 13, 2339–2352
11. Vignali, M., Hassan, A. H., Neely, K. E., and Workman, J. L. (2000) Mol. Cell 20, 1899–1910
12. Lorch, Y., Cairns, B. R., Zhang, M., and Kornberg, R. D. (1998) Cell 94, 29–34
13. Schnitzky, G., Sif, S., and Kingston, R. E. (1998) Cell 94, 17–27
14. Bazett-Jones, D. P., Clote, J., Landel, C. C., Peterson, C. L., and Workman, J. L. (1999) Mol. Cell Biol. 19, 1470–1478
15. Hamische, A., Sandaltzopoulos, R., Gedula, D. A., and Wu, C. (1999) Cell 97, 833–842
16. Længst, G., Bonte, E. J., Corona, D. F. V., and Becker, P. B. (1999) Cell 97, 843–852
17. Lorch, Y., Zhang, M., and Kornberg, R. D. (1999) Cell 96, 389–392
18. Whitehouse, I., Flaus, A., Cairns, B. R., White, M. F., Workman, J. L., and Owen-Hughes, T. (1999) Nature 400, 784–787
19. Jakubiec, A., Gein, I. M., Peterson, C. L., and Logie, C. (2000) Mol. Cell Biol. 20, 3588–3598
20. Shen, C.-H., Leblanc, B. P., Alifier, J. A., and Clark, D. J. (2001) Mol. Cell Biol. 21, 534–547
21. Butt, T. R., Sternberg, E. J., Gorman, J. A., Clark, P., Hamer, D., Rosenberg, M., and Crooke, S. T. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 3332–3336
22. Karin, M., Najarian, R., Haslinger, A., Valenzuela, A. P., Welch, J., and Fogel, S. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 337–341
23. Fürst, P., Hu, S., Hackett, R., and Hamer, D. (1988) Cell 55, 705–717
24. Buchman, C., Skroch, P., Welch, J., Fogel, S., and Karin, M. (1989) Mol. Cell Biol. 9, 4091–4095
25. Cullotta, V. C., Hu, S., Fürst, P., and Hamer, D. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 8377–8381
26. Leblanc, B. P., Benham, C. J., and Clark, D. J. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 10745–10750
27. Yenidunya, A., Davey, C., Clark, D. J., Felsenfeld, G., and Allan, J. (1994) J. Mol. Biol. 237, 401–414
28. Clark, D. J., and Felsenfeld, G. (1991) EMBO J. 10, 387–395
29. Studitsky, V. M., Clark, D. J., and Felsenfeld, G. (1994) Cell 76, 371–382
30. Meersseman, G., Penninga, S., and Bradbury, E. M. (1992) EMBO J. 11, 2951–2959
31. Clark, D. J., and Felsenfeld, G. (1992) Cell 71, 11–22
32. Davey, C., Penninga, S., Meersseman, G., Wess, T. J., and Allan, J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 11210–11214
33. Davey, C., Penninga, S., and Allan, J. (1997) J. Mol. Biol. 267, 276–288
34. Snyder, M., Buchman, A. R., and Davis, R. W. (1986) Nature 324, 87–89
35. Simpson, R. T. (1986) Bioessays 4, 172–176
36. Rhodes, D. (1985) EMBO J. 4, 3473–3482
37. Thoma, F., and Simpson, R. T. (1985) Nature 315, 250–252
38. Dong, F., Hansen, J. C., and van Holde, K. E. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 5724–5728
39. O’Donohue, M. F., Duband-Goulet, I., Hamiche, A., and Prunell, A. (1994) Nucleic Acids Res. 22, 987–995
40. Buttinelli, M., Di Mauro, E., and Negri, R. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 9315–9319
41. Zhang, X.-Y., and Hirz, W. (1984) J. Mol. Biol. 176, 105–129
42. Linxweiler, W., and Horz, W. (1985) Cell 42, 281–290
43. Perlmann, T., and Wrange, O. (1988) EMBO J. 7, 3073–3079
44. Piña, B., Bruggemeier, U., and Beato, M. (1990) Cell 60, 719–731
45. Roberts, M. S., Fragos, G., and Hager, G. L. (1995) Biochemistry 34, 12470–12480
46. Fragos, G., John, S., Roberts, M. S., and Hager, G. L. (1995) Genes Dev. 9, 1933–1947
47. Buckle, R., Balmer, M., Yenidunya, A., and Allan, J. (1991) Nucleic Acids Res. 19, 1219–1226
48. McPherson, C. E., Shim, E., Friedman, D. S., and Zaret, K. S. (1993) Cell 75, 387–398
49. Shim, E., Woodcock, C., and Zaret, K. S. (1998) Genes Dev. 12, 5–10
50. Belikov, S., Gelius, B., Almouzni, G., and Wrangle, O. (2000) EMBO J. 19, 1023–1033
51. Rainbow, M., Lopez, J., and Lohr, D. (1989) Biochemistry 28, 7486–7490
52. Ura, K., Kurumizaka, H., Dimitrov, S., Almouzni, G., and Wolffe, A. P. (1997) EMBO J. 16, 2096–2107
53. Fras, A., and Richmond, R. J. (1998) J. Mol. Biol. 275, 427–441
54. Spadafora, C., Ouédraogo, P., and Chambon, P. (1979) Eur. J. Biochem. 100, 225–235
55. Drew, H. R., and Travers, A. A. (1985) J. Mol. Biol. 186, 773–790
56. Puhl, H. L., and Behe, M. J. (1985) J. Mol. Biol. 245, 559–567
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J. Biol. Chem. 2001, 276:35209-35216.
doi: 10.1074/jbc.M104733200 originally published online July 18, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M104733200

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