A RSC/Nucleosome Complex Determines Chromatin Architecture and Facilitates Activator Binding

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SUMMARY

How is chromatin architecture established and what role does it play in transcription? We show that the yeast regulatory locus UASg bears, in addition to binding sites for the activator Gal4, sites bound by the RSC complex. RSC positions a nucleosome, evidently partially unwound, in a structure that facilitates Gal4 binding to its sites. The complex comprises a barrier that imposes characteristic features of chromatin architecture. In the absence of RSC, ordinary nucleosomes encroach over the UASg and compete with Gal4 for binding. Taken with our previous work, the results show that both prior to and following induction, specific DNA-binding proteins are the predominant determinants of chromatin architecture at the GAL1/10 genes. RSC/nucleosome complexes are also found scattered around the yeast genome. Higher eukaryotic RSC lacks the specific DNA-binding determinants found on yeast RSC, and evidently Gal4 works in those organisms despite whatever obstacle broadly positioned nucleosomes present.

INTRODUCTION

“Chromatin architecture” refers, generally, to the disposition of nucleosomes along DNA molecules in a population of cells. The classical approach to determine nucleosome positioning is to digest chromatin with micrococcal nuclease (MNase) such that the primary product comprises mononucleosomes and then to identify the protected DNA fragments. Only nucleosomes (and not, for example, the transcription complex; Bryant et al., 2008) protect segments of DNA in this assay, and the recovered nucleosomal fragments usually span about 150 bp. When populations of yeast cells are analyzed in this way, recurrent features of chromatin architecture are observed at regions in and around promoters (reviewed in Jiang and Pugh, 2009; Rando and Chang, 2009). These features include, in addition to nucleosomes positioned more or less at random, “phased” nucleosomes that occupy identical positions on DNA throughout the population; 100–300 bp segments that bear no nucleosomes (called nucleosome-free regions, NFRs); 10–20 bp segments that register as hypersensitive sites (HS’s); and, sometimes, nucleosomes containing H2A.Z, a variant of the more common nucleosome subunit H2A. What determines nucleosome identities and positions, and to what end?

A partial answer to these questions has come from studies of certain inducible genes in yeast. In these cases, one or another transcriptional activator effects removal of nucleosomes that form in adjacent promoter regions prior to induction. This reaction clears the way for subsequent recruitment by the activator of the transcriptional machinery, and absent this step, induction is delayed (Bryant et al., 2008; Korber and Horz, 2004). For example, upon induction of either the PHO5 or GAL1/10 genes, a DNA-bound transcriptional activator (Pho4 in one case and Gal4 in the other) recruits the “nucleosome remodeler” Swi/Snf, which rapidly removes nucleosomes lying adjacent to the site of binding of each activator. NFRs of 100–300 bp are thus created upon induction of these genes. It has been suggested that another member of the Swi/Snf family, RSC, can also be recruited to DNA by specific DNA binding proteins (Badis et al., 2008 and Hartley and Madhani, 2009; see also Ng et al., 2002 and Parnell et al., 2008). Unlike Swi/Snf, RSC bears specific zinc-cluster DNA-binding determinants (Angus-Hill et al., 2001), and it has been suggested that RSC, either recruited to DNA by another protein or binding DNA on its own, removes nucleosomes (Badis et al., 2008; Hartley and Madhani, 2009). RSC, as we shall describe, plays an important role at the GAL1/10 genes, but not by removing nucleosomes.

These findings left open the question of what role chromatin architecture might play prior to induction. For example, do nucleosomes compete with regulatory proteins (e.g., Gal4) for binding to DNA, and if so, how significant is that effect and how might it be overcome or avoided? One possibility is that DNA sequences in eukaryotes have evolved with differing nucleosome-forming propensities, and the sites of binding of regulatory proteins are maintained relatively nucleosome-free. Were this the case, one
would expect that reconstitution experiments with purified histones and DNA would produce a distribution of nucleosomes that would leave critical sites unoccupied. Contrary to this expectation, it was reported (in contrast to an earlier claim; Terrell et al., 2002) that reconstitution of such a nucleosome pattern at the yeast PHO5 gene requires, in addition to histones, one or more unidentified proteins in a cell extract (Korber and Horz, 2004). Genome-wide nucleosome reconstitution experiments with yeast DNA have not settled the problem; two recent studies differ significantly in the reported degree to which nucleosomes reconstituted in vitro occupy positions similar to those found in vivo (Kaplan et al., 2009; Zhang et al., 2009). The problem is of general interest in view of the fact that Gal4 is a “universal” activator. That is, when ectopically expressed, it can activate any of a wide array of genes in higher eukaryotes modified so as to bear Gal4-binding sites nearby.

We recently described a quantitative MNase-protection assay that reveals not only nucleosome positioning but also, especially for well-positioned nucleosomes, the fraction of the population that bears a protecting nucleosome for any given position and instant (Bryant et al., 2008). In outline, we measure the nuclease sensitivity of each of a wide array of ca. 60 bp segments (amplicons). The typical DNA fragment yields a biphasic curve, indicating the presence of two populations: one that is highly sensitive (as though it were naked) and the other that is highly protected (as though it bears a nucleosome). These curves differ in their inflection points and thus reveal the fraction of templates in the population, for any given segment, that bear a nucleosome. Rarely, curves are seen that indicate that every member of the population is naked (hypersensitive, HS) or, in contrast, is occupied.

We applied this method to analyzing the chromatin architecture prior to and following induction of the yeast GAL1/10 genes (Bryant et al., 2008 and see Results, Figure 1A). One striking finding was that the UASg, which bears Gal4-binding sites, behaved differently than did any other DNA segment in the region. First, it was protected by some unknown factor in 100% of the population, and second, the protected segment was some 30 bp shorter than that protected by a typical nucleosome. Previous studies had variably suggested that the UASg is nucleosome free, that it bears a nucleosome, and/or that it bears some unusual factor (Bryant et al., 2008; Cavalli and Thoma, 1993; Fedor and Kornberg, 1989; Fedor et al., 1988; Kaplan et al., 2009; Lee et al., 2007; Lohr, 1984, 1993).

Here we show that a complex comprising RSC and a nucleosome is bound constitutively, and independently of Gal4, to the UASg. The unusually small size (for a nucleosome) of the protected UASg DNA fragment(s), confirmed here by paired-end DNA sequencing (Illumina), reflects, we suggest, the presence of a partially unwound nucleosome. The complex, placed in an ectopic position, suffices to impose characteristic features of chromatin architecture found at the GAL1/10 locus—including phased nucleosomes and hypersensitive sites—on the flanking DNA. We attribute this effect to the tight positioning of the complex imposed by specific binding of RSC to sites in the UASg. Removal of RSC from the UASg (effected either by inactivation of RSC or by deletion of a segment of the UASg) allows general encroachment of nucleosomes over the locus. Absent RSC at the UASg, Gal4 binding is impeded (but not blocked), a result indicating that the RSC/nucleosome complex presents the Gal4 sites in the UASg for ready access. The loss of this complex at the UASg has biological consequences under at least one physiological condition. A preliminary survey reveals the presence of RSC/nucleosome complexes scattered throughout the yeast genome. The RSC found in higher eukaryotes, so far as we know, lacks the specific DNA-binding determinants found on yeast RSC (Mohrmann and Verrijzer, 2005), and, consistent with nucleosome disposition on a UASg inserted into a mammalian cell as reported here, evidently Gal4 works in such cells against whatever obstacle broadly positioned nucleosomes might present.

RESULTS

Figure 1A shows the chromatin architecture at the GAL1/10 locus as found in wild-type cells determined using the method of Bryant et al. (2008). Cells were grown in the absence of galactose, and so the GAL genes were silent. Rather precisely positioned nucleosomes (one to the left and two to the right in the figure) flank the UASg. The boundaries of the UASg are marked by short (ca. 10–20 bp) HS’s. As indicated in the figure, no more than 5% of these HS sequences in the population are occluded by nucleosomes. The regions separating flanking nucleosomes from each other are also unusually sensitive to the nuclease, but less so than the HS sites (20% versus 5% of the population protected), and so are labeled hs. The repeat length of the nucleosomes, measured as the distance between the centers of hypersensitive sites, is about 165 bp. This is as expected if the core nucleosome includes the typical 147 bp with adjacent nucleosomes separated by about 18 bp of linker DNA. Each of these nucleosomes, however, fully protects less than 50% of the population at any given instant, a finding consistent with the observation that promoter nucleosomes in yeast tend to exchange more rapidly than do other nucleosomes (Dion et al., 2007; Linger and Tyler, 2006).

In contrast, and as indicated in the figure, some unknown factor protects the UASg in virtually every member of the population. The protected region encompassing the UASg spans only some 135 bp measured as the distance between the centers of the flanking HS sites. We also found that the MNase-protection pattern observed for wild-type (WT) cells is unaltered by deletion of gal4 (see Figure S1 available online). It was reported that insertion of the UASg into a plasmid caused phased nucleosomes to form adjacent to the UASg (Fedor and Kornberg, 1989). A mutational analysis showed that Reb1, a protein thought to bind the UASg, was not responsible for this effect (Reagan and Majors, 1998). What then is the factor that binds to and strongly protects the UASg from nuclease digestion, and what is its physiological role?

RSC Bound to the UASg

Figure 1B displays DNA fragments bearing both a nucleosome (as indicated by protection from MNase digestion) and RSC (as indicated by the presence of the TAP-FLAG). For this experiment, chromatin was digested under conditions that yielded primarily mononucleosomes, and RSC-bearing fragments were...
recovered on IgG-beads. Fragments (of size ca. 50–200 bp) were analyzed by paired-end high-throughput DNA sequencing (Illumina). This technique determines the sequences found at both ends of each fragment, thus revealing the sizes and genomic origins of these fragments. Figure 1B shows the number of sequenced fragments that cross any given base pair (i.e., the “fragment density”) along the region between the \( \text{GAL1} \) and \( \text{GAL10} \) genes. The figure shows a strikingly well-positioned peak over the \( \text{UASg} \).

Figure 1C shows in a different way that RSC is bound at the \( \text{UASg} \) and also shows that this binding depends upon the integrity of one of its DNA-binding subunits. The mutation \( \text{rsc3-1} \) renders heat sensitive the Rsc3 subunit, which bears one of RSC’s putative DNA-binding zinc clusters (Angus-Hill et al., 2001). For this experiment, cells bearing TAP-tagged RSC containing the \( \text{rsc3-1} \) mutation were grown at various temperatures as indicated, chromatin subjected to MNase digestion and immunoprecipitated as in Figure 1B, and RSC-bearing fragments characterized by real-time quantitative PCR (QPCR). The figure shows that the peak of RSC at the \( \text{UASg} \) is diminished as the cells are grown for longer times at the nonpermissive temperature. Figure 1D shows the result of an experiment.
performed like that of Figure 1C, except that the cells were deleted for gal4, bore a WT RSC fused to the TAP-tag, and were grown at 30°C. The figure shows that the peak of RSC at the UASg forms independently of Gal4, and its MNase resistance indicates that a nucleosome is also present.

Figure 1E shows that the UASg bears, in addition to four Gal4-binding sites, at least two sites that match the proposed (weak) RSC consensus binding sequence (Badis et al., 2008), and a third that differs at one position. By deleting the sequences to the right of the arrow in the schematic, we generated a UASg bearing the three strongest Gal4-binding sites (sites 1–3) but lacking the putative RSC-binding sites as well as the fourth, weak, Gal4-binding site. The experiment of Figure 1F, performed like that of Figure 1C, shows that binding of RSC was drastically reduced by this deletion.

A “Small,” H2A.Z-Containing Nucleosome at the UASg
Our finding that the UASg was protected from nuclease (MNase) digestion indicates that this DNA, in addition to bearing RSC as just described, is also wrapped in a nucleosome. This surprise was confirmed in the experiments of Figures 2A and 2B. Chromatin digested as in the experiment of Figure 1C was precipitated with antibodies recognizing H2B, H3, and H4, and digested with MNase, and the resulting DNA subjected to paired-end sequencing (Illumina) as described in the legend of Figure 1B. The blue line indicates the density of the resulting MNase-protected fragments over the GAL1/10 locus, and the fragments bound by RSC and protected from MNase digestion of Figure 1B are shown for comparison (red line).

The distributions of the sizes of the mapped fragments at the UASg (left inset) and at the neighboring nucleosome are shown (right inset).

(D) Size distribution of MNase-protected fragments over the GAL1/10 locus. MNase-protected fragments were determined as described in (C) and the number of fragments of the sizes indicated is represented by the color saturation (see Experimental Procedures). The fragment density curve of (C) is superimposed for reference. The boxed areas indicate fragments of sizes corresponding to ordinary nucleosomes—in the ORFs and the one to the right of the UASg (labeled “normal”)—and fragments of smaller sizes that are associated with the RSC/nucleosome complex at the UASg (labeled “smaller”).
contain H2A. Nucleosomes bearing H2A.Z at this site were re-sequenced fragments along the DNA is represented by the blue curve. The density of sequenced fragments along the DNA is represented by the blue curve. The two insets above the curves show the fragment size distributions of two regions of the blue curve: on the left are UASg fragments, and on the right are fragments from the region protected by the nucleosome just to the right of the UASg. The figure shows that the UASg fragments cluster around 120 bp, whereas those at the adjacent site cluster around 150 bp. The difference in size distribution is statistically significant to p < 0.001 as determined by a two-sample Kolmogorov-Smirnov test. Figure 2D shows the fragment size distribution along a large part of the GAL1/10 region. It is readily apparent that the weakly phased nucleosomes in the open reading frames (ORFs) yield protected fragments of sizes similar to those in the promoters, whereas the UASg protected fragments are shorter. The important result is that, as inferred from our earlier studies (Bryant et al., 2008), the RSC/nucleosome complex at the UASg protects fragments that are, on average, 30 bp smaller than those protected by a typical nucleosome. And the finding noted above, that 100% of the UASg’s in the population are fully protected from MNase digestion indicates that every UASg bears a nucleosome that protects the unusually small DNA fragments.

As a side note, we have observed a difference between the MNase-protection patterns at the GAL1/10 locus depending on whether digested chromatin is assayed by QPCR or by Illumina sequencing. Thus the curve generated by Illumina sequencing all genomic fragments are amplified by PCR prior to obtaining sufficient amounts of each fragment for Illumina sequencing.

**The Effect of RSC on Induction of the GAL Genes**

What effect does the RSC/nucleosome complex—with its attendant chromatin architecture—have on regulation of expression of the GAL genes? Figure 4A shows that addition of galactose to cells growing in raffinose induced expression of GAL1 equally quickly, and to the same extent, whether or not a ts RSC was inactivated by growth at the nonpermissive temperature. Figure 4B shows that, consistent with this finding, induction of a strain growing in raffinose and bearing the truncated UASg, which does not bind RSC, followed the same time course as did induction of WT cells. In contrast, when cells were grown in glucose and then transferred to galactose, inactivation of RSC (or elimination of RSC-binding sites) resulted in a marked delay in induction (Figures 4C and 4D). We explain these findings as follows.

It is well known that in cells growing in raffinose, Gal4 is expressed and binds the UASg, but its activating region is covered by the inhibitor Gal80. Addition of galactose inactivates Gal80, thereby freeing Gal4’s activating region and triggering activation of transcription. Glucose, however, represses Gal4 expression (an aspect of the “glucose repression” effect at the GAL genes), and upon transfer to galactose, Gal4 levels must first be increased, and the newly synthesized Gal4 must bind the UASg before it can work. We imagine that in the absence of RSC,
Gal4 must compete with broadly bound nucleosomes for binding to the UASg, whereas in its presence the UASg is held readily accessible to Gal4. According to this idea, for cells growing in raffinose and absent RSC at the UASg, Gal4 would slowly compete away the nucleosomes, bind its sites on the UASg, and thus be positioned to respond rapidly to galactose. In contrast, for identical cells growing in glucose and then transferred to galactose, some delay in induction would be incurred as Gal4 competes for binding to its sites.

These ideas make predictions realized in the experiments of Figures 4E–4H. Figure 4E shows that cells grown overnight in raffinose indeed have Gal4 bound to the UASg whether or not RSC is present. Figure 4F shows that for cells growing in glucose and then transferred to galactose, binding of Gal4 to the UASg is significantly delayed in the absence of RSC. Figure 4G shows that this delay is not accounted for by a delay in expression of Gal4 caused by RSC inactivation. That is, as measured by the levels of GAL4 mRNA, Gal4 is expressed equally well in this scenario whether or not RSC is inactivated. Figure 4H confirms a key aspect of this picture in another way. The fusion protein Gal4-ER-VP16 is held in the cytoplasm in the absence of estrogen, and upon addition of the hormone the fusion protein enters the nucleus and binds the UASg (Nalley et al., 2006). The figure shows that in such an experiment, the fusion protein binds the truncated UASg lacking RSC sites significantly more slowly than it binds a WT UASg.

UASg described in (E) were grown in raffinose and a ChIP experiment detecting Gal4 was performed as described (Floer et al., 2008) except that chromatin was digested with MNase prior to immunoprecipitation. Gal4 binding is shown as fold over a control location in the PHO5 gene.

(G) RSC binding to the UASg inserted at an ectopic position. Cells bearing the ectopically positioned UASg and TAP-tagged RSC were grown in raffinose, chromatin was treated with MNase, and a ChIP experiment recognizing the TAP-tag was performed as in Figure 1C.

Figure 3. The Effect of RSC Binding to the UASg on Chromatin Architecture
(A) Histone H3 binding to the UASg in the presence and absence of bound RSC. Cells bearing rsc3-1ts (a mutation in a DNA-binding subunit) were grown in glucose at 25°C (black) and then shifted to 37°C for the times indicated (red and blue). Cells were probed for histone H3 as described for Figure 2A.

(B) Histone H3 binding to the UASg in the absence of active RSC. Cells bearing RSC s11-3ts (a mutation in RSC's catalytic subunit) grown in glucose at the permissive (black) and nonpermissive (red) temperatures were probed for H3 as in (A).

(C) A MNase-protection experiment was performed as described (Bryant et al., 2008). In brief, the MNase digestion data were subjected to curve fitting by nonlinear least squares analysis and the error represents the deviation from the best fit, when the sum of squares increased by 10% (for details, see Bryant et al., 2008). Cells bearing the rsc3-1ts mutation were grown in raffinose at 25°C and then shifted to 37°C, and MNase protection assayed at various times. The black bar above the schematic shows the position analyzed, and the dots show the increasing protection of the HS site as the cells were grown at 37°C. Similar results were found for the HS site to the right of the UASg in the schematic (not shown).

(D) Effect of truncating the UASg on an HS site. An MNase-protection experiment was performed as in (C) with cells bearing the truncated UASg of Figure 2D.

(E) Nucleosome disposition at and around a UASg inserted at an ectopic position. A MNase-protection experiment was performed using cells bearing a UASg inserted 551 bp downstream from the GAL1 translation start site. In this mutant the DNA spanning the GAL1-GAL10 promoters was deleted. Protection was analyzed after growth of cells in noninducing medium (2% raffinose, blue curve) and 30 min following addition of 2% galactose (red curve). The numbers below the figure describe the percent protection of the hatched bars, indicating that HS sites flanking the UASg were created by the insertion.

(F) Gal4 binding to the UASg inserted at an ectopic position. Cells bearing the ectopically positioned UASg and TAP-tagged RSC were grown in raffinose, chromatin was treated with MNase, and a ChIP experiment recognizing the TAP-tag was performed as in Figure 1C.
RSC/Nucleosome Complexes Elsewhere in the Genome

Having surveyed the entire genome we have found some 4100 RSC/nucleosome peaks, listed in Table S1. These peaks were identified by comparing genome-wide Illumina sequencing data from the MNase-protection experiment of Figure 2C with those of the MNase-protection/RSC ChIP experiment of Figure 1B. Where a peak of RSC was greater in the latter data compared with the former, we assigned a location for a RSC/nucleosome complex. Preliminary analysis suggests that the majority of these RSC/nucleosome complexes protect fragments similar in sizes to those protected by an ordinary nucleosome (not shown). But a significant fraction of the complexes protects fragments similar in sizes to those protected by the complex at the UASg. Figure 5 shows four examples from chromosome II (which contains the GAL1/10 locus) of RSC/nucleosome complexes found in or near promoters, all of which yield protected fragments of sizes similar to those seen for the UASg. We do not know the function of any of these RSC/nucleosome complexes found outside the GAL1/10 locus.

A UASg Placed in a Mammalian Cell

The specific DNA-binding determinants of RSC are found in the two subunits Rsc3 and Rsc30 (Angus-Hill et al., 2001). The mammalian homolog of yeast RSC lacks the Rsc3 and Rsc30 subunits (Mohrmann and Verrijzer, 2005; Wilson et al., 2006). We expected, therefore, that a UASg transferred to mammalian cells would lack the characteristic structure found at the UASg in WT yeast and would more closely resemble that seen in a yeast rsc3-1 mutant. This expectation is borne out in the experiment of Figure 6. HeLa cells were transfected with an integrating plasmid bearing a 700 bp region spanning the GAL1/10 promoters and the UASg. MNase-protected chromatin from selected integrants was then probed for histone H2B as in the experiment performed with yeast in Figure 2A. A signal was broadly distributed over the region, similar to that observed for the UASg in yeast in the absence of RSC (data not shown). Consistent with these findings, the MNase-protection experiment of Figure 6 shows that HS sites are not found associated with the UASg inserted into a mammalian genome. Thus both the ChIP and MNase-protection assays indicate a broad distribution of nucleosomes over the UASg in a mammalian cell, similar to that seen in yeast lacking RSC activity.

DISCUSSION

Determinants of Chromatin Architecture at the GAL1/10 Genes

We show that two DNA-binding proteins, each of which recognizes specific sites in the UASg, determine chromatin architecture independent of sequence context. Thus, RSC, which traps an unusual nucleosome on the UASg, establishes chromatin architecture prior to induction, and Gal4, bound to the UASg, directs removal of promoter nucleosomes upon induction. This conclusion implies that different intrinsic nucleosome-forming potentials of different DNA sequences play little role in this architecture. This conclusion might be tempered by the following considerations. First, the regions flanking the UASg are relatively depleted of nucleosomes. That is, although the nucleosomes form at more or less specified (phased) sites, they tend to form less frequently than do typical nucleosomes—those found in ORFs, for example (see Figure 1A and Bryant et al., 2008). It is possible that this relatively low occupancy of predetermined sites reflects the inherent nucleosome-forming propensities of these DNA sequences, a notion consistent with our finding that, when the UASg is positioned at an ectopic site, the flanking phased nucleosomes form more readily than when the UASg is at its wild-type location. Whether DNA sequence plays this role, and what might be its biological significance, remains for further investigation. Second, perhaps the UASg spontaneously forms a nucleosome with high frequency in vivo, and perhaps that property helps stabilize the final RSC/nucleosome complex. Experiments performed in vitro indicate that the UASg readily wraps into nucleosomes (Kaplan et al., 2009; Rainbow et al., 1989).

The RSC/Nucleosome Complex and Chromatin Architecture

How does the RSC/"small" nucleosome complex, at the UASg, cause phasing of flanking nucleosomes and the creation of HS sites? The complex is held in a tight position by the specific DNA-binding determinants on RSC. That tight positioning, we imagine, presents a barrier that excludes nucleosomal encroachment (Kornberg, 1981). Such a barrier would tend to cause phasing of nucleosomes to be "phased," an effect that would diminish as we move away from the barrier. The hypersensitive sites would also be explained by the barrier effect. Thus every UASg in the population would present an identical barrier, and the inability of nucleosomes to encroach on that barrier would render a short bit of DNA sensitive to MNase in every cell in the population. Such a short sequence would appear as hypersensitive in the MNase-protection assay. Nucleosome phasing and HS sites thus are not determined by the identities of the sequences adjacent to the UASg but rather are a consequence of the barrier effect. A typical nucleosome would not present a well-defined barrier because, unlike a specific DNA-binding protein, a nucleosome, even if bound to a favorable site, will tend to occupy a distribution of sites, which differ modulo 10 bp. This is because DNA, in wrapping around the histone octamer, makes many contacts with the protein (at intervals of some 10 bp), and so ratcheting the nucleosome by 10 bp will have little effect on its stability (see Ioshikhes et al., 2006 for a fuller discussion).

Our experiments raise the possibility that for many genes the presumed role of RSC—i.e., to remove promoter nucleosomes—might usefully be reconsidered. It is reported that mutation of RSC causes an increase in nucleosome density and decreased gene activation at various loci in the genome (Badis et al., 2008; Hartley and Madhani, 2009). A similar finding would apply to the GAL genes, but our analysis shows that at that locus RSC plays no direct role in nucleosome removal. Rather, RSC facilitates activator binding. Furthermore, nucleosome depletion at the GAL locus (low nucleosome occupancy prior to induction and absence of nucleosomes following induction) does not play the role often ascribed to NFRs, i.e., to facilitate activator binding. Rather, the GAL1 and GAL10 NFRs are created by the activator (Gal4 in this case), and there is no obvious way that the depletion of nucleosomes prior to induction could influence Gal4 binding.
Figure 4. Effects of RSC on Induction of GAL1 and on Binding of Gal4 to the UASg

(A) Effect of inactivating RSC on induction: Raff → Gal. Galactose was added to WT (blue) or rsc3-1ts mutant cells (red) growing in raffinose at 37°C for 3 hr. At the times indicated GAL1 mRNA levels were determined as described (Floer et al., 2008).

(B) Effect of deleting putative RSC binding sites on induction: Raff → Gal. Galactose was added to raffinose-grown cells that bore WT RSC and either the WT (blue) or the truncated UASg (red) of Figure 2D.

(C) Effect of inactivating RSC on induction: Glu → Gal. WT (blue) and rsc3-1ts cells (red) were grown in glucose for 3 hr at 37°C and then shifted to media lacking glucose but containing galactose and raffinose. GAL1 mRNA levels were measured as described in (A).

(D) Effect of removing RSC-binding sites on induction: Glu → Gal. An experiment was performed like that described in (C) except that cells bearing WT RSC and either the WT (blue) or truncated UASg (red) were grown at 30°C.

(E) Effect of inactivating RSC on Gal4 bound to the UASg: Raff. WT cells (blue) and rsc3-1ts cells (red) were grown in raffinose at 25°C and then shifted for 3 hr to 37°C. Gal4 binding was analyzed as described for Figure 3F and is shown normalized to a control locus in the PHO5 gene.

(F) Effect of inactivating RSC on Gal4 binding to the UASg: Glu → Gal. WT (blue) and rsc3-1ts cells (red) were grown in glucose for 3 hr at 37°C and shifted to medium containing galactose. Gal4 binding to the UASg was analyzed as described in (E).
A “Small” Nucleosome

The “paired-end” DNA sequencing technique (Illumina), which determines sequences from both ends of each sequenced fragment, confirms our earlier surprising finding that the size of the UASg fragment protected from MNase digestion is about 30 bp smaller than that protected by the typical nucleosome. Moreover, as we show here, the protecting factor includes RSC plus all four histones found in a nucleosome. These results indicate that the UASg is wrapped in a nucleosome complexed with RSC in such a manner that the DNA is partially unwound from the histone octamer. MNase digestion experiments can readily miss the presence of “smaller” nucleosomes such as that found at the UASg. Thus if one assumes that nucleosomes protect DNA of size 150 bp and first isolates such sized fragments prior to further analysis, the shorter protected regions would be missed. Another way that shorter fragments might be overlooked would be by analyzing protected fragments by any method that sequences just one end of any individual fragment and assuming the location of the other end based on the usual size of sequences protected by nucleosomes.

The predominant form of the H2A subunit at the UASg is the minor variant H2A.Z. Perhaps this subunit interacts more efficiently with RSC than does the major H2A species. If so, the preference is not absolute, as indicated by the fact that in a strain deleted for H2A.Z, the RSC nucleosome complex at the UASg forms (Figure S2). Other experiments have suggested a relation between H2A.Z incorporation into chromatin and RSC (Hartley and Madhani, 2009).

RSC/Nucleosome Complexes Elsewhere in the Genome

We have detected some 4100 RSC/nucleosome peaks along the S. cerevisiae genome using the criteria described in the Extended Experimental Procedures. Preliminary analysis indicates that some 5%–20% of these complexes protect fragments shorter than those associated with ordinary nucleosomes. These RSC/“small” nucleosome complexes are overrepresented in or near promoters (not shown); we show four examples in Figure 5. Other RSC/nucleosome complexes, found more commonly in ORFs, protect fragments of sizes expected to be protected by ordinary nucleosomes (not shown). It is possible that different positioning of RSC-binding sites produces these different structures. These and related matters remain for further investigation.

Role of Chromatin Architecture in Gene Regulation

Our results indicate that the RSC/partially unwound nucleosome complex facilitates Gal4 binding to its sites in the UASg, and this

Figure 5. “Small” Nucleosomes Associated with RSC at Various Locations in the Genome

The distribution of fragments protected from MNase digestion at four promoters found on chromosome II. The data are displayed as in Figure 2D, except that the disposition of RSC (taken from data of the experiment of Figure 1B) is overlayed in red. The names of the genes and the coordinates along the genome are shown in the figure.

(G) Effect of inactivating RSC on GAL4 mRNA production. GAL4 mRNA levels were measured for cells grown as described for the experiment of (C).

(H) Effect of deleting RSC-binding sites on binding of a hormone regulated Gal4-fusion protein to the UASg. Cells were deleted for gal4 but contained a plasmid expressing a myc-tagged Gal4DBD-ER-VP16 fusion (Nalley et al., 2006), and either the WT UASg (blue) or its truncated derivative (red). Gal4 binding to the UASg was determined at the times indicated, following addition of estrogen to cells growing in glucose, by probing for myc. The data were normalized to a control locus in the PHO5 gene.
slower binding has a physiological consequence when cells grown in glucose are transferred to galactose. In this scenario newly made Gal4 more rapidly binds the UASg and induces transcription, if RSC is present at the UASg. Thus the RSC/partially unwound nucleosome complex, bound at the UASg, would confer a significant growth advantage to yeast on the assumption that a rapid response to the environmental change (glucose to galactose) can be important.

The fact that the RSC found in higher eukaryotes lacks the DNA-binding determinants of yeast RSC, and our finding that (as therefore expected) a UASg placed in a mammalian cell bears only broadly positioned nucleosomes (Figure 6), suggests the possibility that in such organisms there will be a necessary delay between the introduction of Gal4 and the activation of transcription of the target gene. We imagine that the speed with which this will happen could well depend upon the concentration of Gal4, the number of binding sites present in synthetic UASg’s, and so on. How such considerations might apply to other activators and genes in yeast and higher eukaryotes remains to be seen.

A Model for The RSC/Nucleosome Complex at the UASg

Figure 7A shows a model for a RSC/nucleosome complex (Chaban et al., 2008), based on cryo-EM and biochemical studies of a RSC/mononucleosome complex (Asturias et al., 2002; Lorch et al., 2001). In this model, RSC engulfs the nucleosome and partially unwinds it, contacting but not entirely covering the DNA remaining on the histone octamer surface. Inspired by this model, we projected a 118 bp UASg onto a sphere the size of a nucleosome (Figure 7B). In the projection, the center of the UASg is aligned with the dyad axis of the nucleosome, and 80 bp of the UASg wraps around the histone octamer. Comparison of Figures 7A and 7B suggests that the putative RSC-binding sites in the UASg (red segments) could contact RSC; the Gal4-binding sites (blue) 1 and 4 would lie in the unwrapped portion of the DNA; and Gal4-binding sites 2 and 3 would lie on the surface of the nucleosome not covered by RSC. Figure 7C (modeled for us by Francisco Asturias) shows that Gal4 (here represented by two dimeric Gal4 DNA-binding domains) could bind sites 2 and 3 without destroying the structure. This model remains speculative at this point because, among other uncertainties, the path of the DNA in the structure of Chaban et al. (2008) is not well defined. Nevertheless the model is strikingly consistent with our results.

The Ubiquity of Inhibitors and Small Effects

In a broader context, our findings illustrate principles that apply to many biological regulatory processes, especially in eukaryotes. First, where those systems are regulated by binding reactions, transcription being a salient example, inhibitors are required to suppress basal reactions. Those inhibitors must be readily overcome when the system is activated. Nucleosomes are widely believed to suppress basal transcription, and we and others have previously shown how promoter nucleosomes can be removed upon command (Bryant et al., 2008; Reinke and Horz, 2003). Second, many regulatory features may be regarded as add-ons that facilitate, but are not absolutely required, for any particular case. The GAL genes show us two examples: recruitment of Swi/Snf and its subsequent action, which facilitates the initiation of transcription as described, is not absolutely required—that reaction occurs in the absence of Swi/Snf, but more slowly (Bryant et al., 2008). A similar description of the RSC/partially unwound nucleosome complex would seem to apply here as well—its presence facilitates Gal4 binding to the UASg upon induction but is not absolutely required. And, it seems likely that in the many cases where ectopically expressed Gal4 is used to express heterologous genes in higher eukaryotes, it does so in the absence of the facilitating RSC/partially unwound nucleosome complex. Add-ons, such as the RSC/partially unwound nucleosome complex, are found widely in biology, and they make systems that work, work better. Put another way, the prevalence of machinery with small effects illustrates the power of natural selection (Ptashne, 2009).

EXPERIMENTAL PROCEDURES

Yeast Strains and Growth Conditions

The strains and plasmids used in the experiments as well as the growth conditions are listed in the Extended Experimental Procedures. In general yeast cells were grown exponentially in SC media containing 2% of the sugars indicated.
Mammalian Plasmid Construction and Cell Transfection

The plasmid used for integrating the yeast UASg into mammalian chromatin was generated by inserting a 790 bp fragment containing the GAL1/10 regulatory region and the initial base pairs of the GAL1 and GAL10 ORFs into the pAcGFP1-1 vector (Clontech Laboratories, INC). The plasmid was integrated into HeLa cells using Fugene 6 (Roche Applied Science, Indianapolis, IN, USA) and integrants were selected.

ChIP Experiments and mRNA Determination

ChIP experiments probing for RSC, histones, or Gal4 were performed essentially as described (Floer et al., 2008) except that where indicated crosslinked ChIP experiments probing for RSC, histones, or Gal4 were performed essentially as described (Bryant et al., 2008). For experiments with mammalian chromatin, MNase-protection experiments of yeast chromatin were performed as described (Bryant and Ptashne, 2003) or by paired-end high-throughput sequencing (Illumina) as described below. GAL1 and GAL4 mRNA was assayed as described (Floer et al., 2008).

MNase-Protection Experiments

MNase-protection experiments of yeast chromatin were performed as described (Bryant et al., 2008). For experiments with mammalian chromatin, clones bearing the integrated UASg were selected and the MNase digestion experiments were performed with six individual clones with essentially identical results. The exact sequences of the primers used can be given upon request.

Paired-End DNA Sequencing (Illumina)

For these experiments, cells were crosslinked with formaldehyde, sonicated, and treated with MNase as described for the ChIP experiments (see above). The resulting MNase-protected fragments were purified (QIAGEN) without further size-separation. (Inspection of the resulting fragments by agarose gel electrophoresis revealed fragments of sizes 50–200 bp.) For the analysis of fragments bound by RSC MNase-protected DNA (from cells bearing TAP-tagged RSC) was precipitated on IgG-beads, followed by QIAGEN purification. A detailed description of the paired-end sequencing (Illumina) method and of the analysis to determine RSC-bound peaks can be found in the Extended Experimental Procedures. In brief, a DNA library for paired-end sequencing was created and the sequencing data were processed on an Illumina GA analysis pipeline. Reads passing Illumina quality filters were mapped to the S. cerevisiae genome obtained from the Saccharomyces Genome Database (http://www.yeastgenome.org/) on July 24, 2009. The 36 base-paired end reads were mapped using MAQ alignment software with default settings (Li et al., 2008). The fragment density maps of Figure 1B and Figures 2C and 2D were generated by calculating the number of fragments that cross any given base pair along the genome. The fragment size distributions (histograms of the insets in Figure 2C) were generated by calculating the number of fragments that cross the range of DNA indicated by the gray box. The fragment size distribution along the genomic position (in modules of 8 bp), the numbers of fragments of each size and the color saturation representing the average fragment count (taken from Chaban et al., 2008). RSC interaction of the UASg onto a nucleosome. The UASg was modeled onto a single turn of a nucleosome (corresponding to 80 bp) with the dyad axis placed in the center of the UASg. Gal4 sites are shown in blue and putative RSC-binding sites in red. Alignment of this projection with the structure in (A) places Gal4 sites 1 and 4 on the unwrapped ends of the nucleosome and sites 2 and 3 on a part of the nucleosome that is largely accessible in the structure. The positions of the putative RSC-binding sites in the UASg correspond closely to the three RSC densities shown to contact nucleosomal DNA.

(C) Model of Gal4 binding to sites 2 and 3 in a UASg bound by a RSC/nucleosome complex. The UASg was positioned in the RSC/nucleosome structure of (A) as described in (B). Two Gal4 dimers (as represented by their DNA-binding and dimerization domains, shown in red and purple) were positioned on Gal4-binding sites 2 and 3. The orientation of Gal4 dimers on DNA was taken from the Gal4/DNA structure of Hong et al. (2008). The model shows that sites 2 and 3 are exposed along a surface of the RSC/UASg/nucleosome complex and that Gal4 can bind these sites without disrupting the structure.

ACCESSION NUMBERS

The GEO accession number for the genome-wide datasets is GSE20078.
SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, two figures, and one table and can be found with this article online at doi:10.1016/j.cell.2010.03.048.

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Supplemental Information

EXTENDED EXPERIMENTAL PROCEDURES

Yeast Strains and Plasmids
The rsc3-1ts strain (bearing the mutant originally obtained by Brad Cairns) and its derivative containing the TAP-tagged Rsc8 subunit, as well as a strain bearing WT RSC3 and the TAP-tagged Rsc8 subunit, were provided by Timothy Hughes (Badis et al., 2008). The sth1-3ts strain was provided by Brehon Laurent (Du et al., 1998). The strain expressing myc-tagged histone H4 was constructed by introducing a plasmid bearing the tagged histone (Keener et al., 1997) into BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0). The strain bearing FLAG-tagged histone H2B was similarly constructed from introducing a plasmid bearing tagged HTB1 into BY4741 (Sun and Allis, 2002). The strain bearing HA-tagged H2A as the sole copy of H2A was a gift from Francesc Posas, at Pompeu Fabra University (Barcelona).

To construct the mutant strain that bears a truncated UASg a two-step process was used (Storici et al., 2003). In brief, a cassette containing the Kan’ and URA3 genes was first inserted in place of the GAL1/10 UASg and subsequently replaced with the sequence of the mutant UASg. The same method was also used to construct the mutant strain in which the 118 bp fragment encompassing the UASg was inserted into the GAL1 ORF 551 bp downstream from the translational start site. (In this strain the endogenous GAL1/10 regulatory region had been deleted by the same method.) Subsequently the TAP-tag was fused to the C terminus of RSC8 in each of these strains by the method of (Ghaemmaghami et al., 2003). The sequences of the oligonucleotides used for the construction of these strains can be given upon request.

The gal4 deleted strain bearing WT RSC was a derivative of BY4741 obtained from EUROSCARF (European Saccharomyces Cerevisiae Archive for Functional Analysis). The gal4 deleted strains bearing the Gal4-ER-VP16 fusions were constructed by deleting the GAL4 ORF in the BY4741 WT background or in the derivative that bears the truncated UASg using the PCR based method of Brachmann et al. (1998). The plasmid used for expression of Gal4DBD-ER-VP16 and obtained from Tom Kodadek (Nalley et al., 2006) was introduced into these strains. GAL4 was also deleted in the TAP-tagged RSC8 strain from Tim Hughes.

Growth Conditions
Yeast cells were grown logarithmically in SC media containing 2% glucose or 2% raffinose as indicated in the figure legends. For experiments involving temperature shifts cells were grown at 25° C and then the temperature was increased to 37° C on a heat plate, followed by growth of the cells at 37° C for the indicated times. For induction experiments of cells pre-grown in glucose cells were collected by centrifugation and directly resuspended in fresh media containing 2% galactose and raffinose each. The experiments assaying for Gal4 binding were performed by growing cells in SC media containing 2% glucose, and adding estrogen at a final concentration of 50 nM.

ChIP Experiments and mRNA Determination
ChIP experiments probing for Gal4 were performed essentially as described (Floer et al., 2008). For ChIP experiments probing for histone or RSC binding to MNase-protected DNA fragments, formaldehyde crosslinked chromatin was incubated for 90 min at 37° C with limiting amounts of MNase (0.015 U to 0.062 U per 200 µl reactions) prior to incubation with antibodies against histone H3 (Abcam), H2A.Z (from Hiten Madhani), the myc-epitope (Abcam), the FLAG-epitope (Sigma), the HA-epitope (Santa Cruz Biotechnology) or with IgG-Sepharose beads (GE Healthcare). The resulting immunoprecipitated DNA was analyzed in two ways: (1) The immunoprecipitated DNA was analyzed by quantitative PCR as described (Bryant and Ptashne, 2003). The percentage of the DNA immunoprecipitated in each experiment was normalized to a location in the PHOS promoter, except for the experiment determining RSC binding in the ts rsc3-1 mutant strain, where unnormalized percent IP (immunoprecipitated DNA) is shown. The exact sequences of the primers used in these experiments can be given upon request. (2) The RSC bound DNA was analyzed by paired-end high-throughput sequencing (Illumina) as described below. To determine GAL1 or GAL4 induction RNA was extracted, reverse transcribed and the resulting cDNA was assayed with primers corresponding to locations in the GAL1 or GAL4 gene and control genes as described (Floer et al., 2008).

Normalization of Histone ChIP of MNase-Protected DNA Fragments
In a previous analysis (Bryant et al., 2008) we had incorrectly surmised that the protection at the UASg could not be due to the presence of a nucleosome because of the smaller size of the protected DNA fragment, and we presented a faulty ChIP experiment (recognizing histone H2B) indicating the absence of histones at the UASg. Our initial ChIP analysis was wrong due to incorrect normalization, a danger, we now believe, of assaying protection of short fragments that lie between two strong hypersensitive sites. When properly normalized, as in the ChIP experiments of Figures 2A and 2B, the presence of four different histones is clearly evident.

Mammalian Plasmid Construction and Cell Transfection
The yeast GAL1/10 regulatory region including the UASg and the initial base pairs of the GAL1 and GAL10 ORFs (encompassing a 790 bp fragment) was cloned into the pAcGFP1-1 vector (Clontech Laboratories, INC). Human HeLa cells were cultured in Dulbecco’s modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS), 100 µg/ml penicillin, and 100 U/ml streptomycin. HeLa cells were plated at 1 x 10^5 cells per 10 ml in 10 cm tissue culture dishes one day prior to transfection. Cells were transfected
using Fugene 6 (Roche Applied Science, Indianapolis, IN) per manufacturer’s instructions. The transfected cells were maintained in selection media containing 400 μg/ml G418 (Invitrogen, Carsbad, CA).

### MNase-Protection Analysis of Mammalian DNA

For each experiment 2 × 10^7 cells were cross-linked for 10 min by adding formaldehyde directly to the growing culture at a final concentration of 1%. The reaction was stopped by addition of 0.125 M glycine, cells were washed twice with PBS and the assays were performed essentially as described for experiments performed with yeast cells except that 5.6 μl of 10 mM CaCl_2 was added to each 200 μl reaction mixture to start the MNase digestion reaction. The digested DNA was analyzed by quantitative PCR as described (Bryant et al., 2008).

### Creation of the DNA Libraries for Paired-End Illumina Sequencing

End repair and phosphorylation of purified DNA was performed in a 100 μl reaction containing 500 ng of DNA as template, 1 × T4 DNA Ligase Buffer with 10 mM ATP (NEB), 0.4 mM each of dATP, dTTP, dCTP, dGTP (Roche) in the presence of 0.15 U/μl T4 DNA Polymerase (NEB), 0.05 U/μl Klenow DNA Polymerase (NEB), and 0.5 U/μl T4 Polynucleotide Kinase (NEB). The reaction was incubated at 20°C for 30 min then purified using QIAquick PCR Purification (QIAGEN) following the manufacturer’s instruction. For 3’ A-overhang addition the eluted DNA was treated in a 50 μl reaction with 0.3 U/μl Klenow Fragment (NEB) in 1 × Buffer 2 (NEB) and 2 mM dATP (Promega) for 30 min at 37°C. After the purification using MinElute PCR Purification (QIAGEN) following the manufacturer’s instructions, the ends of the eluted DNA were ligated to the Illumina paired-end adapters (PE5’Adaptor and PE7’Adaptor) using Quick Ligation (NEB). The ligation reaction contained 10:1 molar ratio of adapters to genomic DNA insert and was carried out at room temperature for 15 min then purified using QIAquick PCR Purification (QIAGEN). 150–300 bp fragments were selected on a 2% agarose gel after running for 60 min at 100 V and purified by Gel Extraction (QIAGEN). Finally, the DNA library was enriched by PCR using Phusion High Fidelity PCR Master Mix (NEB) and 0.1 μM each of paired-end primers PE5’ and PE7’. Cycling conditions were as follows: (1) 98°C for 30 s, (2) 12 cycles of 98°C for 10 s, 65°C for 30 s, and 72°C for 30 s, (3) a final extension of 5 min at 72°C. After purification of the PCR reactions through QIAquick PCR columns (QIAGEN), the eluted DNA library was loaded on an Illumina GA2 flow cell as per manufacturers instructions.

### Identification of Candidate RSC/Nucleosome-Bound Peaks

We identified candidate loci bound by RSC and also protected against MNase by comparing the fragments resulting from paired-end sequencing of the chromatin that had been MNase digested and purified on IgG-beads to immunoprecipitate RSC (IP set), with those resulting from sequencing of chromatin that had only been digested with MNase (BG set). Although the two datasets were derived from separate experiments the second dataset represents a good approximation to the background of fragments protected against MNase prior to RSC immunoprecipitation. We developed a statistical algorithm to compute, for each genomic position, its probability to be enriched in the IP set compared to the BG set. Candidate RSC bound peaks were identified as closely spaced groups of genomic positions with a high probability.

Our algorithm is closely related to the pinning algorithm (A.K. and J.H., unpublished data), originally designed to detect loci of significantly recurrent aberration in array-CGH data. The algorithm consists of two parts. In the first part the number (L) of such loci was determined recursively as follows (see Box 1). Beginning with the IP set, we found at each step a locus with a maximal fragment count. This maximal count is our statistic. Its null distribution was obtained by drawing at random, for each fragment in the IP set, a fragment of the same length from the BG set. The maximal fragment count was computed for the resulting random fragment set. This procedure was repeated multiple times to yield the null distribution of maximal counts. If the null hypothesis was rejected, the fragments containing the optimal locus were removed from both the IP and the BG sets, and the entire step was repeated for the remainder of these sets.

We determined the likely positions of the recurrent loci taking into account errors in both directions in the inclusion of fragments in the IP fragment set. To this end we computed, for each base pair in scope, its probability to belong to a significant recurrent locus. This was done by generating multiple bootstrap samples of S_p and finding the L recurrent loci in each sample, as detailed in Box 2.

We applied the two-part procedure as described to the RSC IP and BG fragment sets for all chromosomes. A significance level $p_{max} = 0.05$ (see Box 1) was required for a locus to be considered recurrent. We obtained, for each genomic position X, its probability $P(X)$ to belong to a recurrent locus. Where multiple positions with $P(X)$ above 0.05 were found within 100 bp of each other, these positions were considered to be members of the same peak. Peaks are identified by their location and the maximum probability value in Table S1.

### SUPPLEMENTAL REFERENCES


Box 1. Algorithm for finding the number of significantly recurrent loci that are bound by a RSC/nucleosome complex

// \text{S}\text{P} and \text{S}\text{BG} are the IP and BG fragment sets of a chromosome. \text{P} (0<\text{P}<1) is the // significance threshold. \text{K} is the number of random draws for testing the null hypothesis

FUNCTION count_recurrent_loci(\text{S}\text{P}, \text{S}\text{BG}, \text{P}\text{max}, \text{K})
\begin{align*}
\text{s}\text{IP} &\leftarrow \text{S}\text{IP} \quad //\text{initialize the IP remainder} \\
\text{s}\text{BG} &\leftarrow \text{S}\text{BG} \quad //\text{initialize the BG remainder} \\
\text{p} &\leftarrow 0 \quad //\text{initialize the p value} \\
\text{L} &\leftarrow 0 \quad //\text{initialize the number of recurrent loci} \\
\text{WHILE} (\text{p} < \text{P}\text{max} \text{ AND } \text{s}\text{IP} \text{ is not empty} \text{ AND } \text{s}\text{BG} \text{ is not empty}) \\
\text{N} &\leftarrow \text{maximal } \text{s}\text{IP} \text{ fragment count anywhere on the chromosome} \\
\text{X} &\leftarrow \text{a base in the overlap of } \text{N} \text{ s}\text{IP} \text{ fragments} \\
\text{A} &\leftarrow 0 \quad //\text{the number of times the null hypothesis is accepted in } \text{K} \text{ draws} \\
\text{FOR} i \text{ in } 1,2,...,\text{K} \\
\text{s} &\leftarrow \text{empty set} \\
\text{FOR each fragment in } \text{s}\text{IP} \\
\text{s} &\leftarrow \text{s} + \text{(a fragment of equal length drawn at random from } \text{s}\text{BG}) \\
\text{END FOR} \\
\text{n} &\leftarrow \text{maximal } \text{s} \text{ fragment count anywhere on the chromosome} \\
\text{IF} \text{n} \geq \text{N} \quad \text{A} &\leftarrow \text{A} + 1 \\
\text{END FOR} \\
\text{p} &\leftarrow \text{A}/\text{K} \\
\text{IF} \text{p} < \text{P}\text{max} \quad \text{L} &\leftarrow \text{L} + 1 \\
\text{remove from } \text{s}\text{IP} \text{ fragments containing } \text{X} \\
\text{remove from } \text{s}\text{BG} \text{ fragments containing } \text{X} \\
\text{END WHILE} \\
\text{RETURN } \text{L}
\end{align*}
Box 2. Algorithm for estimating the position-dependent probability of belonging to a recurrent locus bound by a RSC/nucleosome complex

// B is the number of bootstrap samples to generate
FUNCTION recurrent_locus_probability(SIP, L, B)
   FOR all bases X on the chromosome C(X) ← 0 // initialize the fragment count
      FOR i in 1,2,...,B
         s ← bootstrap sample of SIP
         FOR J in 1,2,...,L
            N ← maximal s fragment count
            Z ← (a non-empty intersection of the N intervals in s; if there are several, choose
            one at random)
            FOR all X in Z
               C(X) ← C(X) + 1
            END FOR
            Remove from s all intervals intersecting at Z
         END FOR
      END FOR
      FOR all X
         P(X) ← C(X)/B
      END FOR
   RETURN P(X)
Figure S1. Chromatin Architecture in and around the UASg in the Absence of Gal4, Related to Figure 1
MNase-protection pattern at the UASg in gal4 deleted cells. A MNase-protection experiment was performed with WT cells (blue) and cells deleted for gal4 (EURO SCARF) (red) as described (Bryant et al., 2008), and chromatin architecture was analyzed in and around the UASg. Cells were grown at 25°C in medium containing raffinose.
Figure S2. Chromatin Architecture in and around the UASg in the Absence of the Histone H2A Variant H2A.Z, Related to Figure 2

MNase-protection pattern at the UASg in cells deleted for H2A.Z. A MNase-protection experiment was performed with WT cells (blue) and cells deleted for htz1 (EUROSCARF) (red), which encodes H2A.Z in S. cerevisiae, and protection over the UASg and flanking sequences was determined. Cells were grown at 25°C in medium containing glucose.