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Selective Removal of Promoter Nucleosomes by the RSC Chromatin Remodeling Complex

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Summary

Purified chromatin rings, excised from the *PHO5* locus of yeast in transcriptionally repressed and activated states, were remodeled with RSC and ATP. Nucleosomes were translocated, and those originating on the promoter of repressed rings were removed, whereas those originating on the open reading frame (ORF) were retained. Treatment of the repressed rings with histone deacetylase diminished the removal of promoter nucleosomes. These findings point to a principle of promoter chromatin remodeling for transcription, that promoter-specificity resides primarily in the nucleosomes, rather than in the remodeling complex that acts upon them.

Introduction

Wrapping promoters in nucleosomes prevents the initiation of transcription in vitro[1,2] and in vivo[3]. The nucleosome serves as a general gene repressor, preventing the expression of all genes in eukaryotes except those whose transcription is brought about by positive regulatory mechanisms. It was at first thought that repression by nucleosomes is overcome by the complete removal of histones from promoter DNA. Support for this idea came from the association of transcriptionally active promoters with DNase I hypersensitive sites[4], and from the excision of active promoters with restriction endonucleases as naked DNA[5]. With the advent of chromatin immunoprecipitation, it emerged that histones remain associated with active promoters, but in extensively modified forms (reviewed in ref. 6). This and other evidence led to the view that histones are not entirely displaced from promoter DNA, but rather the nucleosome is altered in structure in some manner susceptible to nuclease attack and conducive to transcription. Clarification came from the isolation of genes from yeast as chromatin in both repressed and transcriptionally active states. The *PHO5* gene of the yeast *Saccharomyces cerevisiae*, in particular, had previously been shown to undergo a marked increase in exposure to nucleases and also increased association with modified histones upon transcriptional activation[7,8]. Isolation of *PHO5* chromatin, by excision from chromosomes in circular form, revealed both the removal and retention of nucleosomes in

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the activated state[9]. Two of the original three promoter nucleosomes were removed, but a single nucleosome was invariably conserved upon activation[10]. Rather than view promoter chromatin remodeling in terms of histone removal or retention, it is more appropriate to describe promoter activation as a transformation from a static to a dynamic state. Upon activation, promoter nucleosomes are rapidly removed and also reassembled, resulting in steady state levels of nucleosomes that vary among promoters and from one position in a promoter to another. Genome-wide mapping of nucleosomes has supported the generality of these conclusions[11,12].

The machinery for mobilizing promoter nucleosomes includes chromatin remodeling complexes, a broad family whose founding member, the SWI/SNF complex, was discovered by genetic studies in yeast. The most abundant family member, termed RSC, is essential for cell growth[13]. Mutations in Swi/Snf proteins interfere with transcription of many genes, and may be suppressed by mutations in histone genes[14]. Direct evidence for interaction with nucleosomes came from isolation of the SWI/SNF and RSC complexes[13,15,16] and demonstration that they expose nucleosomal DNA to nuclease attack in an ATP-dependent manner[13,17,18,19]. It is noteworthy that the SWI/SNF complex exhibits promoter specificity *in vivo*, affecting a particular subset of yeast genes and perturbing promoter but not adjacent nucleosomes. Nevertheless, the complex isolated from yeast was active on virtually any nucleosome, irrespective of DNA or histone source. The reason is that the purified complex could be added in excess to nucleosomes in vitro, obviating any requirement for recruitment by an activator protein or the like. The activity of the remodeling complex could therefore be studied without reconstitution of the entire regulatory pathway, and important insight was gained into the remodeling mechanism.

Although chromatin remodeling complexes exhibit great diversity in size and subunit structure, all contain a conserved catalytic subunit, a nucleic acid-dependent ATPase, and all share a common remodeling mechanism. The ATPases are DNA translocases, which draw DNA in from one side of the nucleosome and expel it from the other[20,21]. As first shown for the SWI/SNF complex and for the closely related RSC complex, DNA within a nucleosome is exposed in an adjacent linker region and made available to attack by nucleases in this way. Structural studies have shown that RSC binds the nucleosome in a central cavity, largely surrounding the particle[22]. RSC binding even in the absence of ATP releases the DNA at both ends of the particle from its tight association with the histone octamer core[23], which facilitates translocation, and which may enable interaction with sequence-specific regulatory proteins in vivo.

Evidence has been presented in some cases for the recruitment of SWI/SNF complex to promoters by enhancer-binding proteins, explaining the remodeling of active genes, but the basis of specificity for the promoter region, has yet to be established. Why are promoter nucleosomes removed, whereas nucleosomes upstream and nucleosomes associated with the ORF downstream are unaffected? Is promoter-specificity attributable to the enhancer, or is it a property of the promoter sequence, or does it reside elsewhere? We have investigated this question with RSC for remodeling and with purified *PHO5* chromatin circles as substrate. As in previous studies of SWI/SNF and RSC action on isolated nucleosomes, we employed RSC in excess, to avoid the requirement for targeting by an activator protein. This strategy

led to the surprising finding that specificity for remodeling resides in the promoter nucleosomes.

Results

RSC redistributes nucleosomes in purified *PHO5* chromatin rings

PHO5 gene and promoter chromatin rings, containing 2200 and 750 bp of DNA, respectively (Supplementary Fig. 1), were purified 180,000-fold with respect to other chromosomal loci[24]. When isolated in the transcriptionally repressed state, both gene and promoter rings bore three positioned nucleosomes on promoter sequences, designated N-1, -2, and -3 [24,25], Supplementary Fig. 1), with an additional nine nucleosomes on the ORF. For purposes of a control described below, chromatin rings devoid of the promoter, containing only the ORF, were prepared as well (Supplementary Fig. 2). Action of RSC on purified rings was studied in the absence of an activator protein (Pho4 protein for the *PHO5* gene) for the reason mentioned above, and because RSC is in any case not responsible for remodeling the *PHO5* promoter *in vivo* (see below). Treatment with RSC and ATP enhanced cleavage of the rings by restriction endonucleases. Sites in nucleosomes, such as the Cla I site in N-2, were largely protected in the repressed rings and almost completely cleaved in the presence of RSC and ATP (Figure 1, lanes 4, 5). Sites in linker regions between nucleosomes, such as the BstE II site between N-1 and N-2, were largely exposed in the repressed state and even more available for cutting in the presence of RSC (Figure 1, lanes 1, 2). In all, enhancement of cutting was seen at seven of eight sites tested in repressed gene rings, and four of five sites in repressed promoter rings (compare first two bars of each set in Figure 2; numerical values under “r”).

When RSC action was terminated by the destruction of ATP with hexokinase and glucose, the enhancement of restriction endonuclease digestion was much diminished (compare last two bars of each set in Figure 2; numerical values under “r”). Sites in nucleosomes, however, such as the Cla I site, remained somewhat more exposed after treatment was terminated than before treatment, while sites in linker regions, such as the BstE II site, were more protected after treatment than before. Evidently, the locations of nucleosomes are altered by RSC treatment. For example, nucleosomes on some rings have vacated the Cla I site, and nucleosomes on these or other rings have come to occupy the BstE II site. As shown previously for nucleosomes assembled *in vitro* with heterologous histones, RSC is evidently capable of DNA translocation and sliding of histone octamers in native chromatin, assembled *in vivo*, containing homologous histones. At the end of the reaction, nucleosomes are “randomized”: nucleosomes that originated on the promoter may have been relocated to the ORF, and *vice versa*; and DNA sites previously protected are exposed, whereas sites previously exposed are protected.

The effect of RSC treatment on restriction endonuclease digestion was also investigated for gene and promoter rings in the transcriptionally activated state (Figure 2, “a”). The accessibility to digestion in the absence of RSC conformed well with the previous results of others[9,26], which have been attributed to the loss of nucleosomes N-1, N-2 and N-3. Treatment with RSC and ATP enhanced the accessibility of all sites, and following termination of the reaction, sites that were initially relatively exposed, such as the Cla I site

and the BstE II site, became more protected, while sites that were initially protected, such as the BspH I site, became more exposed.

The redistribution of nucleosomes on repressed and activated rings following RSC treatment, revealed by restriction endonuclease cleavage at specific sites, was pursued by digestion with micrococcal nuclease, which reveals nucleosomal arrays. Treatment with RSC and ATP disrupted the arrays, as shown by the conversion of the characteristic ladder of bands to a continuous distribution (Supplementary Figs. 2, 3). These findings reinforce the conclusions from restriction analysis: despite differences in posttranslational modifications of the histones[8], RSC is capable of DNA translocation and sliding of histone octamers in both repressed and activated states.

RSC treatment of repressed rings: topological analysis

In previous studies of *PHO5* gene and promoter rings, we employed topological analysis as a quantitative measure of chromatin structure change. A linking difference, ΔLk, of 1.85 was measured between gene rings extracted from repressed and induced cells[9]. Treatment of repressed rings with RSC and ATP also produced a linking difference, revealed upon relaxation with topoisomerase (Figure 3A, compare lanes 4 and 5 with lane 1). The linking difference attained a stable limiting value of about 2.0 with increasing concentration of RSC (lanes 4 and 5 and data not shown). The linking difference imparted by RSC and ATP was irreversible: it persisted following the removal of RSC with competitor DNA and the destruction of ATP by hexokinase and glucose (Figure 3A, lanes 6 and 7). As a control to assure that the amount of competitor DNA was sufficient to remove RSC, the DNA was added before rather than after the reaction. With the levels of RSC used here, the inhibition of the linking change effected by RSC due DNA added before was 55–60% (Figure 3A, lanes 2 and 3), compared with an effect of 5% or less when added after. Although the removal of RSC may not have been complete, the linking change was not due to RSC binding alone.

Relaxation of promoter rather than gene rings in the presence of RSC and ATP resulted in a ΔLk of 0.60 (Figure 3B, lane 1). The effect of RSC and ATP was irreversible, with the ΔLk value actually increasing slightly to 0.76 with the addition of hexokinase and glucose, and to 1.02 with the addition of competitor DNA after the reaction (Figure 3B, lanes 3 and 4). The results were similar to ΔLk values for promoter rings due to transcriptional activation *in vivo*, 0.8 from a previous study (H. Boeger, unpublished data), and 1.1 determined here (Figure 3B, lanes 1 and 5). The action of RSC and ATP upon gene and promoter rings *in vitro* therefore produces the same topological effect as transcriptional activation of the *PHO5* gene *in vivo* (Table 1).

Finally, we examined the effect of RSC and ATP on the topology of activated promoter rings. A ΔLk of 0.49 was obtained (Figure 3B, lane 6), which was almost unaffected by the addition of competitor DNA, hexokinase and glucose after the reaction (lanes 7 and 8). Gel mobility shift analysis showed that competitor DNA completely removed RSC from promoter rings (Supplementary Fig. 4, lanes 2, 3, 6, and 7), so the effect on ΔLk could not be attributed to any residual association with RSC, but rather must be explained by a persistent change in state of the rings.

RSC treatment of repressed rings: nuclease digestion analysis

As previously noted[9], linking differences of chromatin rings such as those reported here may be explained by a persistent change in nucleosome structure, with partial unraveling of the nucleosomal DNA, or by complete unfolding of nucleosomes and the removal of histone octamers from the DNA. These alternatives can be distinguished by limit micrococcal nuclease digestion analysis[9]. In the limit of extensive digestion, the ratio of DNA remaining between two states (rings from activated and repressed cells, in previous work; rings treated with RSC and ATP or untreated, in the present work) is equivalent to the starting ratio of nucleosome core particles between the two states. Multiplying the ratio by the starting number of nucleosomes (12 for gene rings and 3 for promoter rings) and then subtracting from the starting number gives the nucleosome loss. Performing this procedure in the past with gene rings from activated and repressed cells gave a nucleosome loss of 1.9, in good agreement with the result from topological analysis (ΔLk of 1.85)[9]. Performing the procedure with repressed gene rings, treated with RSC and ATP or not, gave a ratio of 0.83 +/− 0.1; with repressed promoter rings, a ratio of 0.58 +/− 0.2; and with repressed ORF rings, a ratio of 1.0 +/− 0.06 (Figure 4). The corresponding values for nucleosome loss were again in good agreement with the results from topological analysis (Table 1). The quantitative agreement between two very different methods of analysis indicates that the results were due entirely to nucleosome loss. Nucleosomes were evidently removed from repressed gene and promoter rings, whereas nucleosomes on ORF rings were unaffected. From the numbers of nucleosomes removed from gene and promoter rings and the absence of effect on ORF nucleosomes, we infer that removal was specific for promoter nucleosomes. (The smaller effect on promoter than on gene rings is discussed below.)

To investigate the basis of specificity for promoter nucleosomes, chromatin rings were treated with histone deacetylase before the addition of RSC and ATP. Nucleosome loss was assessed by limit nuclease digestion (Table 2). Specificity was impaired by this treatment, as nucleosome loss from gene and promoter rings was diminished, and nucleosomes on ORF rings, previously unaffected by RSC, were now partially removed as well. Indeed if the result for gene rings is corrected for loss of ORF nucleosomes, then loss of promoter nucleosomes from gene rings is abrogated entirely by deacetylase treatment. As a control against the possibility of a direct effect of the deacetylase, by inhibition of RSC rather than histone deacetylation, we employed a standard assay of RSC action on nucleosomes *in vitro*. The transfer of histones from reconstituted nucleosomes by RSC and ATP to the histone chaperone NAP1[26] was measured in the presence and absence of the histone deacetylase, and no difference was detected (Figure S5).

Discussion

The principal finding from this work is the specific removal of promoter nucleosomes from chromatin rings formed *in vivo*, upon treatment with RSC and ATP *in vitro*. RSC is capable of action upon native chromatin, sliding homologous (yeast) histone octamers, presumably through DNA translocation, as previously shown for remodeling of nucleosomes assembled from heterologous histones. Nucleosome loss is evident from the quantitative agreement of results of topological analysis and limit nuclease digestion, supported by the results of

limited micrococcal digestion and gel electrophoretic mobility shift analysis. Specificity for promoter nucleosomes is indicated by the numbers of nucleosomes removed from gene and promoter rings, by the correspondence with the numbers previously determined for *PHO5* promoter chromatin remodeling *in vivo*, and by the absence of effect upon ORF nucleosomes.

The behavior of promoter rings is anomalous. There is approximate agreement between the topological and limit nuclease digestion analyses (ΔLk 1.0, nucleosome loss 1.3), and good agreement between ΔLk upon RSC treatment *in vitro* and upon activation *in vivo* (1.0 and 1.0), but there is a discrepancy with the topological and limit nuclease digestion results for gene rings (ΔLk 1.9, nucleosome loss 2.0). In previous work[9,27], in which it was established that two nucleosomes are lost from the *PHO5* promoter upon activation *in vivo*, the same discrepancy was observed – values for promoter rings of 0.8 for ΔLk and of 1.5 for nucleosome loss (measured by nuclease digestion), and values for gene rings of 1.85 for ΔLk and of 1.9 for nucleosome loss. Evidently, the loss of two promoter nucleosomes gives rise to lesser quantitative effects than expected for promoter rings, due perhaps to their small size and consequent topological constraint. The measured value of 0.0 for nucleosome loss from ORF rings *in vitro* provides strong confirmation for the interpretation in terms of the removal of nucleosomes from the promoter alone.

RSC is capable of sliding all nucleosomes on gene rings, yet only promoter nucleosomes are removed. As sliding may be extensive, relocating nucleosomes from the promoter to the ORF and *vice versa*, how are promoter nucleosomes distinguished? Evidently the nucleosomes of the repressed *PHO5* promoter are marked in some manner that is conducive to their removal. Histone modifications, histone variants, and nonhistone proteins are among the possibilities. Treatment with a deacetylase diminished the removal of promoter nucleosomes, and had the further unexpected consequence of enhancing the removal of ORF nucleosomes, suggesting that acetylation at various sites can both stimulate and inhibit nucleosome removal. RSC harbors several bromodomains, which bind acetylated lysine residues, promoting gene activation and transcription[28]. Bromodomain interactions may either increase the affinity of RSC for nucleosomes, and thereby increase the efficiency of remodeling, or cause binding in an unproductive orientation, and thereby oppose remodeling. Such interactions will, in any case, only modulate the activity of RSC upon nucleosomes. The discrimination between promoter and ORF nucleosomes observed at *PHO5* and other promoters *in vivo* may involve additional factors, such as histone variants and nonhistone proteins. The Rsc3 subunit is capable of sequence-specific DNA interaction, which may play a role in remodeling of many yeast promoters as well[29,30].

RSC is unlikely to be responsible for removing nucleosomes from the *PHO5* promoter *in vivo*[31]. Rather, the removal of promoter nucleosomes by RSC from *PHO5* observed here *in vitro* may reflect a general property. Many yeast promoter nucleosomes are inherently unstable due to their content of the histone variant Htz1, an ortholog of mammalian H2AZ. Exposure of yeast chromatin to slightly elevated ionic strength removes Htz1, and presumably the associated H2B, without effect on the other histones[32]. Htz1 is present at the *PHO5* promoter, as well as GAL1-10 and many other promoters in the repressed state[32,33]. Its presence correlates with acetylation of H3 and H4[32]. A double mutant in Htz1 and a

remodeling complex confers an activation defect, and Htz1 is partially lost from promoters in the activated state. These findings, together with the instability of Htz1 nucleosomes, have led to the idea that Htz1 creates a “poised” state of promoter chromatin, prone to nucleosome loss upon recruitment of a remodeling complex[32].

The molecular mechanism of nucleosome removal by RSC may be a common property of remodeling complexes. RSC surrounds the nucleosome to which it is bound and so cannot remove it[22]. By DNA translocation and consequent sliding, however, a RSC-nucleosome complex may invade an adjacent particle and unravel it[34,35]. Inasmuch as Htz1-H2B dimers occupy the entry/exit points of DNA in the nucleosome, they are well placed to facilitate the process. All remodeling complexes translocate DNA, and those that unravel adjacent nucleosomes should exhibit promoter specificity in the remodeling process.

Methods

Restriction endonuclease digestion

PHO5 chromatin rings (25–50 amol), purified as described[24], were digested with 20–40 u of restriction endonuclease in 35 μl of 20 mM HEPES-KOH, pH 7.4, 80 mM potassium acetate, 5 mM $MgCl_2$, 0.8 mM EDTA, 0.1 mM spermine, 0.2 mM spermidine, 8% glycerol and 1 μg of a 21-residue oligonucleotide to suppress secondary site cleavage by some enzymes[24], for 1 h at 30°C. RSC (0.4 μg, purified as described[13]) and ATP (final concentration of 6 mM) were added where indicated. For reactions terminated by the destruction of ATP, an initial incubation was performed with RSC and without restriction enzyme, for 30 min at 30°C, followed by incubation with hexokinase (40 μg, Sigma) and glucose (20 mM final concentration) for 20 min at 30°C, and finally restriction enzyme digestion for 1 h at 30°C. For control reactions in which ATP was destroyed before the addition of RSC, ATP, hexokinase, and glucose were added to the reaction and incubated for 20 min at 30°C, followed by the addition of RSC, incubation for 30 min at 30°C, addition of restriction enzyme, and incubation for 1 h at 30°C. Following restriction enzyme digestion, all reactions were stopped by the addition of 13 μl of 50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 20 mM EDTA, 1% SDS, digestion with proteinase K, and phenol extraction. The DNA was then cut with a second restriction enzyme for mapping the cleavage by the first, separated in a 2% agarose gel in TBE buffer, blotted and hybridized with a ^{32}P-labeled probe. Combinations of first and second restriction enzymes and probe (PCR product with primers indicated in Supplementary Fig. 1) for gene rings were: Hae II, Eco RV, primers p58/p59; ClaI, EcoR V, p58/p59; BstE II (N-1/N-2), EcoR V, primers p58/p59; BspH I, Cla I, p15/p31; Hae III, Nco I, p58/p59; Dra I, Cla I, primers p15/p31; BstE II (ORF), Nco I, p58/p59. Combinations for promoter rings were: Hha I, Hpa II, primers p67/p68; Cla I, Hpa II, p14/p69; Hae II, Cla I, p15/p31; Bsp HI, Cla I, p14/p69; Sal I, Cla I, p15/p31. Quantitation was performed with the use of a PhosphorImager and ImageQuant software.

Partial micrococcal nuclease digestion

Purified gene rings (35 amol) were incubated with or without RSC and ATP in the same manner as for restriction endonuclease digestion except in a volume of 25 μl, without 21-residue oligonucleotide, for 30 min at 30°C. Hexokinase (30 μg) and glucose (final

concentration of 20 mM) were added, followed by incubation for 20 min at 30°C. $CaCl_2$ (0.5 μl of 0.1 M), salmon sperm DNA (1 μl of 10 mg/ml) and buffer (80 μl of 10 mM HEPES-KOH, pH 7.4, 5 mM Tris-HCl, pH 7.5, 40 mM potassium acetate, 0.5 mM CaCl2, 0.4 mM EDTA, 0.05 mM spermine, 0.1 mM spermidine, 4% glycerol) were added, and digestion was performed with 150, 750, or 3675 u of micrococcal nuclease (Sigma) for 5 min at 37°C. Digestion was stopped by the addition of 100 μl of 50 mM Tris-HCl pH 8.0, 500 mM NaCl, 20 mM EDTA, 1% SDS, followed by digestion with proteinase K, phenol extraction, electrophoresis in a 1.2 % agarose gel in TBE buffer, blotting and hybridization with a whole gene probe. For indirect end-label mapping, DNA was digested with PflM I before gel electrophoresis and the probe was ^{32}P-labeled Eco RV – PflM I fragment.

Limit micrococcal nuclease digestion

Purified gene rings (75 amol) were incubated with RSC (3 μg), with or without ATP 0.5 mM, in 20 mM HEPES-KOH, pH 7.4, 20 mM potassium acetate, 4 mM $MgCl_2$, 40 μg/ml BSA, topoisomerase I (4 u), 2 mM EGTA, 1 mM EDTA, 0.025 mM spermine, 0.125 mM spermidine, 5% glycerol in a volume of 90μl for 60 min at 30°C. Apyrase (50 u) was added, followed by incubation for 10 min at 30°C. $CaCl_2$ (4μl of 30 mM) and salmon sperm DNA (1 μl of 10 mg/ml) were added, and digestion was performed with 30 u of micrococcal nuclease (Sigma) for the times indicated at 37°C. Digestion was stopped by the addition of 100 μl of 50 mM Tris-HCl pH 8.0, 500 mM NaCl, 20 mM EDTA, 1% SDS, followed by digestion with proteinase K, phenol extraction, electrophoresis in a 1.5 % agarose gel in TBE buffer, blotting and hybridization with a whole gene probe. For testing the effect of histone deacetylase, HDAC6 (0.4 μg, Cayman Chemical) was included in the initial incubation with RSC.

Electrophoresis of chromatin rings

Promoter rings were incubated with or without RSC and with or without ATP in the same manner as for restriction endonuclease digestion except in a volume of 25 μl, without 21-residue oligonucleotide, for 20 min at 30°C. An unrelated bacterial plasmid DNA (0.3 μg) was added, followed by incubation for 5 min at 30°C and electrophoresis in a 0.8% agarose gel in 0.5xTBE buffer, blotting and hybridization with a ^{32}P-labeled whole circle probe.

Topological analysis

Chromatin rings were incubated with or without RSC and ATP in the same manner as for restriction endonuclease digestion except in a volume of 25 μl, without 21-residue oligonucleotide, for 20 min at 30°C. An unrelated bacterial plasmid DNA (0.6 μg) was added before or after incubation, as indicated, with an additional incubation for 5 min at 30°C after. Hexokinase (30 mg) and glucose (final concentration of 20 mM) were added where indicated, followed by an additional incubation for 20 min at 30°C. TrisCl, pH 7.5 (1 μl of 1M), NaCl (0.5 μl of 2 M), and topoisomerase I (3 u) were added, followed by incubation for 1 h at 37°C. Reactions were terminated by the addition of 10 μl of 50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 20 mM EDTA, 1% SDS, digestion with proteinase K, phenol extraction, electrophoresis in a 1.5% agarose gel in TBE buffer containing 15 μg/ml chloroquine, blotting and hybridization with a whole gene probe. Quantitation was

performed with the use of a PhosphorImager and ImageQuant software. Topoisomer distributions were analyzed and ΔLk values were determined as described[36].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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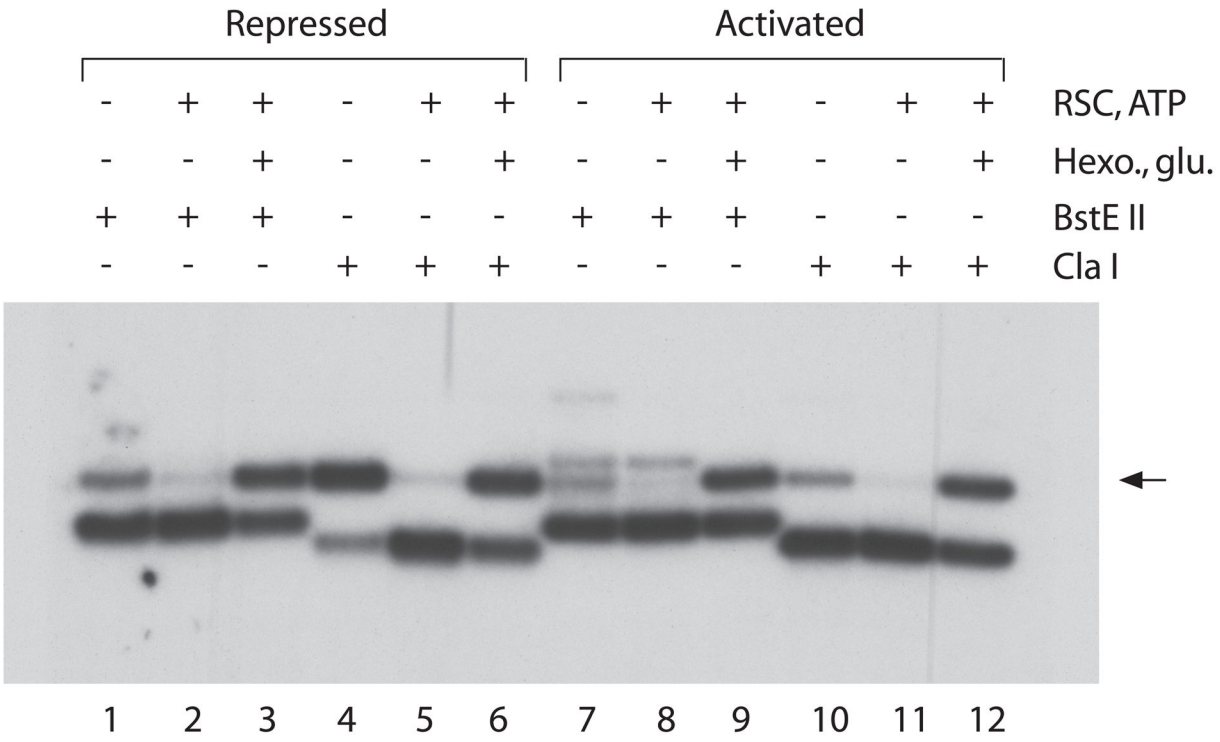

Figure 1.

Effect of RSC and ATP on the accessibility of gene rings to digestion by BstE II and Cla I. ATP was destroyed with hexokinase and glucose after RSC action and before restriction endonuclease digestion in lanes 3, 6, 9, and 12. Blot hybridization was performed with probes described in Experimental Procedures and diagrammed in Supplementary Fig. 1. An arrow indicates the band due to uncut DNA.

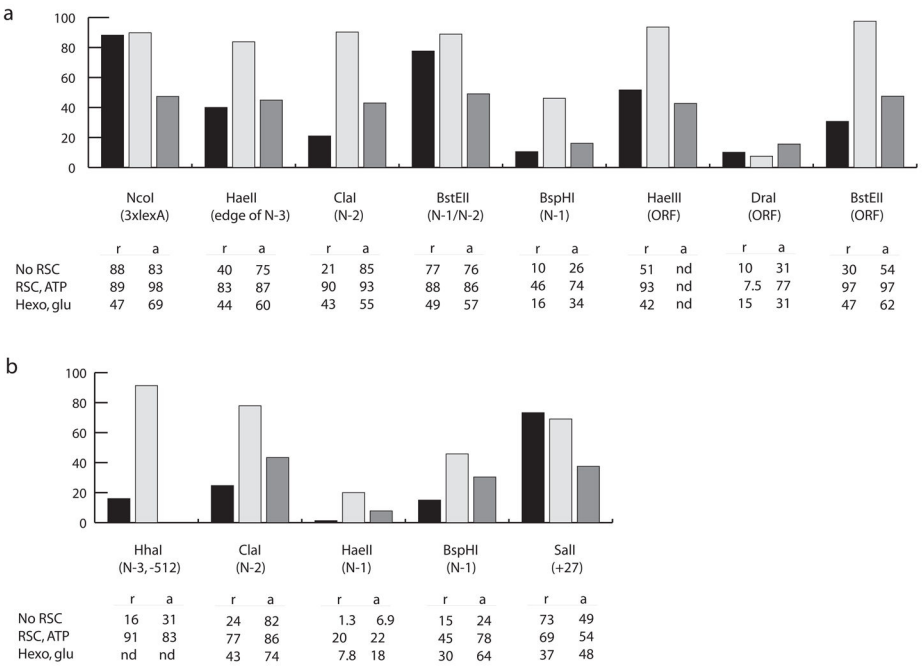

Figure 2.

Effect of RSC and ATP on the accessibility of *PHO5* rings to restriction endonuclease digestion.

(a) Gene rings. **(b)** Promoter rings. The percentages of repressed (r) and activated (a) rings digested are tabulated, and the values for repressed rings are also shown in a bar graph [black bars, no RSC treatment; light gray bars, treatment with RSC and ATP; dark gray bars, treatment with RSC and ATP, followed by destruction of ATP with hexokinase and glucose (hexo, glu)].

Figure 3a

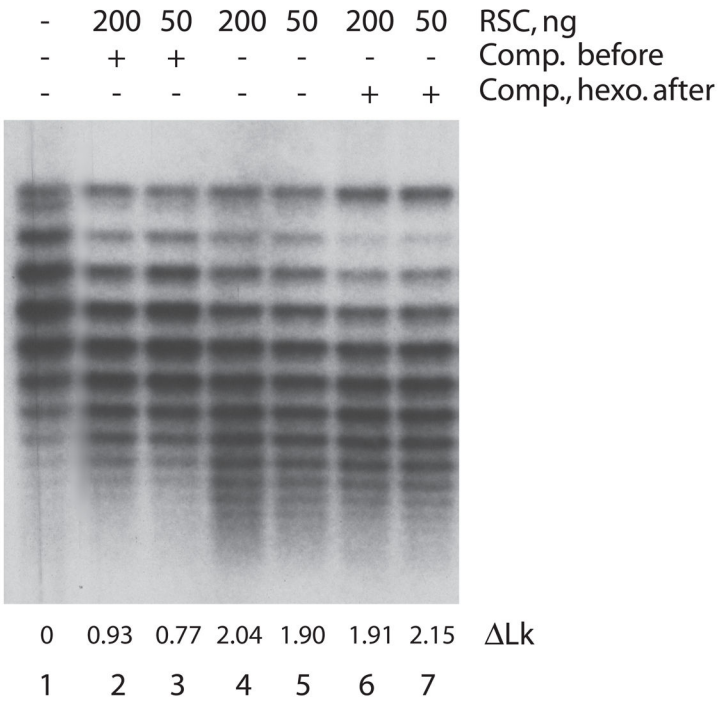

Figure 3b

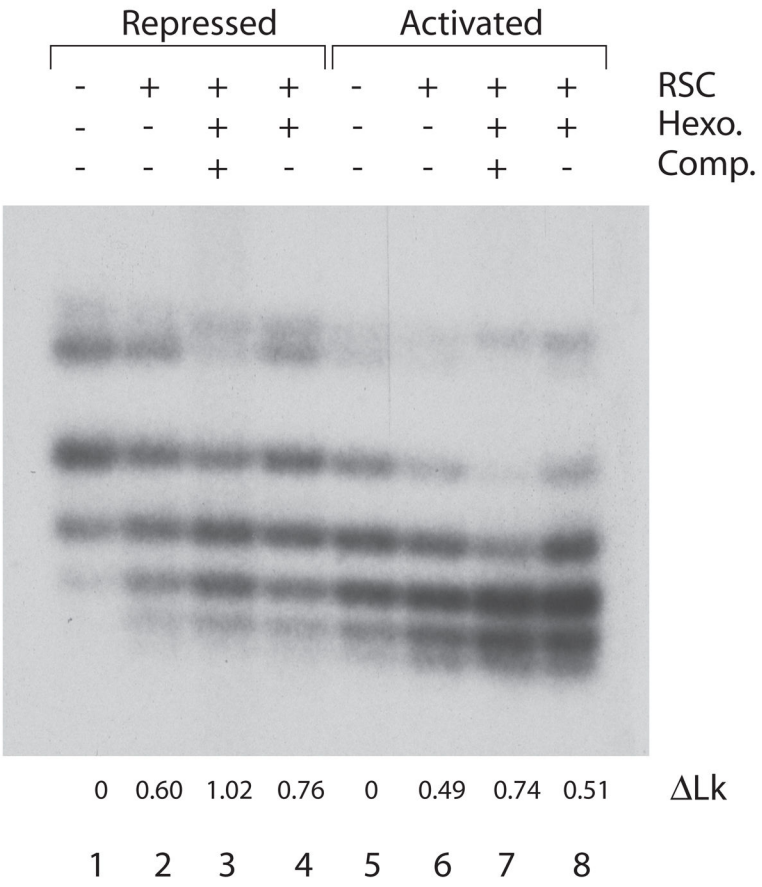

Figure 3.

Effect of RSC and ATP on topoisomer distributions of *PHO5* rings.

(a) Gene rings. **(b)** Promoter rings. RSC was at the level of 200 ng per reaction.

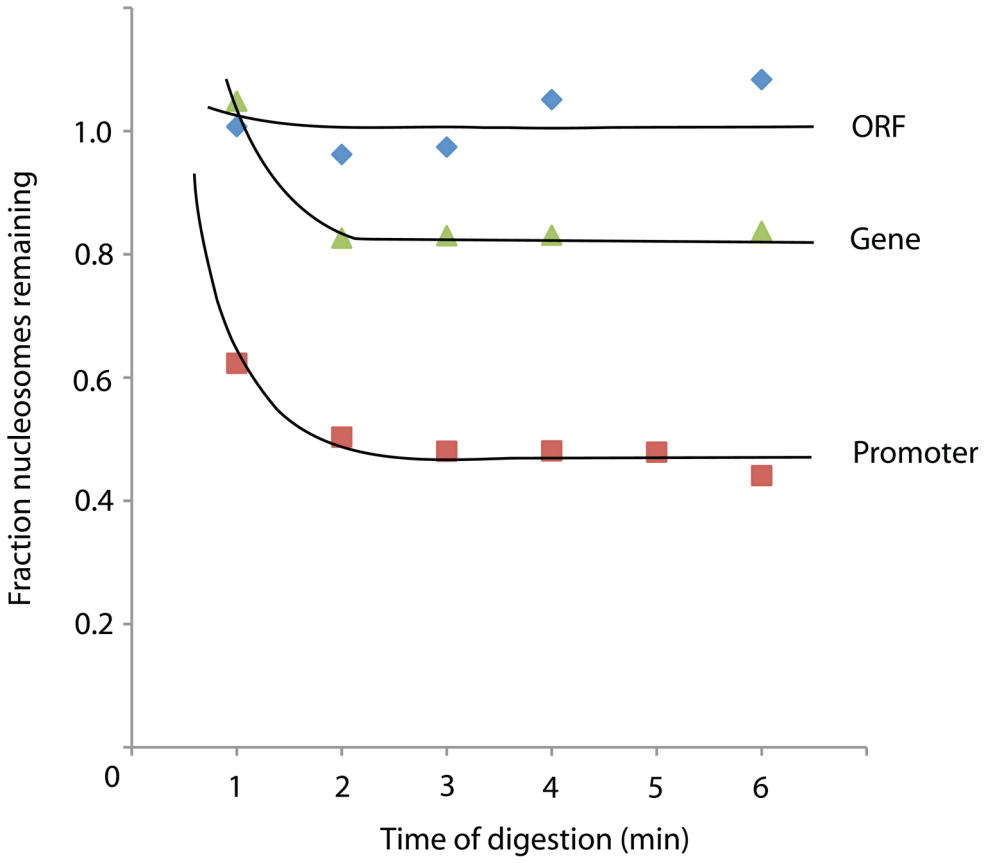

Figure 4.

Nucleosome loss determined by limit nuclease digestion. Examples of limit micrococcal nuclease digestion experiments, performed as described in Experimental Procedures, with calculations as described in Results.

Table 1

Topological change (measured by gel mobility shift) and nucleosome loss (measured by limit nuclease digestion) upon gene activation *in vivo* and upon treatment of chromatin rings with RSC and ATP *in vitro*

Ring	ΔLk *in vitro*	ΔLk, activation *in vivo*	Nucleosome loss *in vitro*
Gene	1.9	1.9	2.0
Promoter	1.0	1.0	1.3
ORF	nd	nd	0.0

Table 2

Nucleosome loss from chromatin rings upon treatment with RSC and ATP in the presence and absence of HDAC6

Ring	− HDAC	+ HDAC6
Gene	2.0	1.1
Promoter	1.3	0.42
ORF	0.0	1.0