Accurate Chromatin Organization of the Mouse Mammary Tumor Virus Promoter Determines the Nature of the Synergism between Transcription Factors*

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The mechanism underlying the synergism between transcription factors in eukaryotic gene expression is not fully understood. In minichromosomes assembled in vitro the synergism between steroid hormone receptors (SHRs) and nuclear factor 1 (NF1) on the mouse mammary tumor virus (MMTV) promoter does not require the proline-rich transactivation domain (PRD) of NF1. Here we show that similar results are obtained in yeast. In contrast, replacing the native hormone-responsive elements (HREs) by a single HRE results in a more accessible chromatin and makes the synergism with SHR dependent on the PRD of NF1. Following hormone induction, in addition to glucocorticoid receptor, the DNA binding domain of NF1 is needed and sufficient for establishing an open chromatin conformation on the wild type MMTV promoter. Thus, NF1 acts as a classical transcription factor in a relaxed chromatin context, whereas in the context of the wild type chromatin DNA binding of NF1 is sufficient to cooperate with SHRs by stabilizing an open chromatin conformation.

Regulation of eukaryotic gene transcription is achieved by combinatorial and synergistic interactions among transcription factors and co-regulators on promoter and/or enhancer regions. Several mechanisms have been claimed to mediate the functional synergism between transcription factors, including cooperative DNA binding, direct interaction between factors, simultaneous interactions with components of the general transcriptional machine or with common co-activators, as well as chromatin-mediated processes. Among the latter there are two main classes of enzymatic mechanisms, ATP-dependent chromatin remodeling (1–3) and histone modifications (4, 5). To analyze the contribution of these different mechanisms in a natural sequence context, we have chosen to study the mouse mammary tumor virus (MMTV) promoter as an example of a promoter induced by steroid hormones (for review, see Ref. 6). The MMTV promoter is organized into positioned nucleosomes and silent in the absence of hormones, while it is rapidly activated in response to glucocorticoids or progestins (7, 8). The promoter encompasses a composite regulatory unit of 148 bp containing five partly degenerated hormone-responsive elements (HREs) upstream of a binding site for nuclear factor 1 (NF1) and two octamer motifs. The HREs are recognized by the receptors for glucocorticoids or progestins, which are ligand-activated transcription factors. The hormone receptors (HRs) interact with the major groove of DNA as homodimers contacting a narrow sector of the double helix (9). NF1 represents a ubiquitous family of transcription factors encoded by at least four different genes, which give rise to several variants due to alternative splicing (10). All variants share a conserved N-terminal DNA binding domain (DBD) and bind as dimers to palindromic sites, completely surrounding the DNA double helix (11). The variants differ in their C-terminal moiety, which has been shown to carry proline-rich transactivation domains (PRDs) (12, 13).

Prior to hormone induction and due to a positioned nucleosome located over the regulatory unit, the NF1 site is not accessible on MMTV chromatin, and only two of the five HREs can be bound by HRs (11, 14). Hormone induction in vivo leads to chromatin remodeling over the regulatory unit manifested by a DNase I hypersensitive site (DHS) (15, 16). This is accompanied by simultaneous occupancy of all the cis-acting elements on the surface of a nucleosome-like particle resulting in functional synergism between the bound factors (8, 17, 18). However, on either naked DNA or MMTV mononucleosomes, HRs do not cooperate with NF1 for binding to their cognate sites in the promoter (14, 19). In addition, the two proteins do not synergize in transcription assays with free MMTV DNA as template (20). As nucleosome depletion eliminates the synergism between receptors and NF1, we have suggested that chromatin is required for synergism and, thus, for efficient activation of the promoter (21). Using MMTV minichromosomes assembled in Drosophila embryo extracts we have shown that chromatin is required for the synergistic binding of progesterone receptor B (PRβ) and NF1 in a two-step process. First, PRβ binds to the accessible HREs and recruits an ATP-dependent chromatin remodeling activity, which transiently opens the chromatin and permits access of NF1 to the promoter. In turn, bound NF1 facilitates the binding of additional PR molecules (22).

To further understand the mechanism of activation of the MMTV promoter we have analyzed the contribution of the PRD of NF1 to the synergism with the glucocorticoid receptor (GR) on the MMTV promoter in Saccharomyces cerevisiae. We have chosen budding yeast because the MMTV promoter behaves in yeast essentially as in mammalian cells, provided the
relevant transcription factors are artificially expressed (23). Moreover, contrary to metazoan cells that are rich in NF1, yeast offers the possibility to manipulate the levels of various NF1 isoforms in the absence of an endogenous homologue. Here we show that DNA binding of NF1 is required for stable hormone-dependent chromatin remodeling over the MMTV promoter. In titration experiments we show that the activity of the MMTV promoter is limited by the amount of ligand-activated GR and not by the levels of NF1. The DNA binding domain of NF1, which is devoid of significant transactivation activity, synergizes with GR in transactivation and chromatin remodeling. Similar results are obtained with MMTV minichromosomes assembled in extracts from Drosophila embryos and transcribed in the presence of PR and either full-length NF1 or the NF1-DBD. In contrast, on a mutant MMTV promoter displaying an open chromatin conformation the synergism between NF1 and GR depends on the transactivation domain of NF1. Thus, the mechanism of synergistic transactivation by hormone receptors and NF1 differs depending on the chromatin organization of the MMTV promoter.

EXPERIMENTAL PROCEDURES

Yeast and Growth Conditions—The yeast strain used in this study was YPH499 (a ade2-101 his3-200 leu2-12 trpl1-1 ura3-52 lys2-801) (24). Growth and manipulation were according to standard procedures (25).

Plasmids—All plasmids were constructed using Escherichia coli strain DH5α. pLGZMMTV (23), pSch105 (26), and pHRE/MMTVv are YEp plasmids based on the URA3 in a fragment inserted at the NF1 site of the MMTV. p415MCTF1 consists of a p415MCTFbd are YCp expression plasmids for pig NF1-C1 and NF1-C2 derived from pAAH5 (28). pAA-CTF (1 plasmid for rat GR (27). pAA-CTF1 is a YEp expression vectors for pig containing exons 1 labeled by T4 polynucleotide kinase and the oligonucleotides 5°/H11032. The restriction enzyme assays were performed essentially as described previously (32) with the following modifications: 50 units of HaeIII were used instead of 200 units, and the second restriction cleavage for the HRE/MMTVv template was performed with PvuII.

RESULTS

Synergism between GR and NF1 Is Limited by the Levels of Activated GR and Is Saturated at Low Concentrations of NF1—The accessibility of the NF1 binding site on the MMTV promoter in yeast is limited by the presence of a positioned nucleosome, which is also required for the synergism between GR and NF1 (21). Removal of the region containing the HREs, as in MMTVΔ (Fig. 1A), makes the NF1 binding site more accessible by destabilizing the positioned nucleosome (26). We used this truncated MMTVΔ promoter to test whether the transcriptional response depends on the levels of NF1 in the cell. Toward this end we expressed NF1-C1 (Fig. 1B) under the control of the MET25 promoter that is regulated by the levels of methionine. This yielded levels of active NF1-C1 that varied by a factor of 15, as determined by GEMSA (Fig. 2C). As expected, transactivation increased linearly with the level of NF1-C1 (Fig. 2A), demonstrating that the PRD is active in yeast and that, within the concentration range tested, there is no other limiting factor. A similar behavior was observed with the wild type MMTV promoter in the absence of ligand, although the absolute values were 10-fold lower and there was no detectable activation at the lowest concentration of NF1 and did not increase further at higher NF1 concentrations. This observation is consistent with the fact that NF1 is bound to the promoter in the absence of ligand, although the absolute values were 10-fold lower and there was no detectable activation at the lowest concentration of NF1 and did not increase further at higher NF1 concentrations (Fig. 2B, circles, left ordinate). The synergism between GR and NF1 was around 6-fold even at concentrations of NF1 that were insufficient for significant activation of the truncated MMTVΔ or the wild type MMTV promoter in the absence of hormone (Fig. 2D). These data suggest that GR-mediated chromatin
remodeling facilitates entry of NF1 and that the affinity of NF1 for the remodeled wild type MMTV chromatin is high compared with its affinity for the nonremodeled wild type MMTV chromatin or the MMTVΔ chromatin.

At lower concentrations of DAC (25 nM), the level of activity decreased around 8-fold. The synergism with GR was also lower but independent of the NF1 concentration (Fig. 2B, triangles, left ordinate, and Fig. 2D, shadowed bars). At higher concentrations of DAC, in the micromolar range, GR alone was already able to activate the MMTV promoter rather efficiently, and the synergism with NF1 was markedly reduced or even insignificant (data not shown). Collectively, our data confirm that the levels of activated GR are the main determinant of the degree of activation of the promoter and that NF1 helps GR only when the activated receptor is present at suboptimal concentrations (22).

**The PRD of NF1 Is Not Required for Synergism with GR on the Wild Type MMTV—NF1 is present in metazoan cells in several variants that all share the N-terminal DNA binding and dimerization domains, whereas their C-terminal half harbors a variable PRD capable of enhancing transcription in yeast, Drosophila, and HeLa cells (12, 13, 33). To directly test the role of the PRD in the synergism with GR on the MMTV promoter, we deleted the whole proline-rich region of NF1 (C319, Fig. 1B). This deletion did not affect the amount of DNA-binding protein, as determined by GEMSA (data not shown). As expected, the deletion mutant showed an insignificant activation of the truncated MMTVΔ compared with the 12-fold activation displayed by the wild type NF1-C1 (Fig. 3A). In contrast, the NF1-C319 mutant exhibited similar synergism with the activated GR as the wild type NF1-C1 protein (Fig. 3B). We have observed a similar synergism with two additional NF1-C variants (C2 and C7) previously shown to transactivate differently in yeast (28) (data not shown). These results strongly suggest that NF1 transactivation is dispensable for synergism with GR. In addition, because the absolute values were very similar for all NF variants and mutants (Fig. 3B and data not shown), one must assume that the PRD of NF1 is not operative in the context of the GR-activated MMTV chromatin.

**The PRD of NF1 Is Required for Synergism with GR on a Mutant MMTV Promoter with an Accessible NF1 Site**—The DNA sequence spanning the five HREs plays a critical role in the maintenance of well organized chromatin structure over the MMTV promoter, as deduced from the increase in NF1 activity and nuclease accessibility observed in the MMTVΔ promoter (Refs. 21 and 26 and Fig. 2). To study the synergism between GR and NF1 in the absence of a regular chromatin organization we cloned a 15-bp consensus HRE just upstream of the NF1 site in the HRE/MMTVA promoter (Fig. 1A, HRE/MMTVA). The activity of NF1 is the same in both MMTVΔ and HRE/MMTVΔ (compare NF1 activities in Fig. 3, A and C, without hormone), suggesting that the NF1 site remains accessible after addition of the single HRE (see below). In the HRE/MMTVΔ promoter, the PRD of NF1 is active in the presence of activated GR, as shown by the decrease in activity caused by its deletion (Fig. 3, NF1-C319). Moreover, in this mutant MMTV promoter there is 4.8-fold synergism between activated GR and NF1, and this synergism requires the PRD of NF1 (Fig. 3C, 100 nM DAC). Thus, the lack of function of the PRD of NF1 in terms of synergism with GR on the wild type MMTV promoter does not reflect an inherent property of the protein or the lack of necessary co-activators, but must be due to the organization of the promoter.
The DBD of NF1 Is Sufficient for Synergism with GR on MMTV—

The N-terminal portion of NF1-C (exons 1–4) is sufficient for site-specific DNA recognition and protein dimerization (13, 34). The PRD-deficient mutant NF1-C319 contains two additional exons coding for a protein sequence that has been claimed to be involved in NF1 transactivation (28). We wondered whether just the DBD of NF1 (C229, Fig. 1B) would be sufficient for the synergism with GR on the MMTV promoter. To test this idea we expressed NF1-C229 using the methionine regulated vector. As shown in Fig. 4A, NF1-C229 does not activate the MMTV promoter in the absence of hormone. Most importantly, NF1-C229 is able to synergize rather efficiently with GR (Fig. 4, A and B). Collectively, our results are compatible with the notion that DNA binding of NF1 to its target site on the MMTV promoter is sufficient for synergism with activated GR in a well organized chromatin context.

NF1 Cooperates with GR in Remodeling MMTV Chromatin, and the DBD of NF1 Is Sufficient for This Effect—In mammalian cells, glucocorticoid induction of the MMTV promoter is known to generate a DHS over the region containing the HREs (8, 16, 35), but the role of NF1 in this process could not be tested due to its ubiquitous expression. To explore whether remodeling of MMTV chromatin can be achieved by activated GR alone or whether NF1 is necessary, we performed in vivo DHS assays in yeast. In the absence of NF1, ligand-activated GR did not generate a DHS over the MMTV promoter (Fig. 5, compare lanes 4 and 5 with lanes 7 and 8). In agreement with the functional data, however, we found a hormone-induced DHS in MMTV chromatin of yeast cells expressing NF1-C2 and GR (Fig. 5, compare lanes 10 and 11 with lanes 13 and 14). Therefore, NF1 is required for efficient remodeling of MMTV chromatin following hormone induction. A similar hormone-dependent change in chromatin structure was observed in cells expressing GR and NF1-C229 (Fig. 5, compare lanes 16 and 17 with lanes 19 and 20). Thus, the DBD of NF1 can fulfill the same function as the natural NF1 variants in terms of cooperating with ligand-activated GR to generate a remodeled chromatin structure over the nucleosome B of the MMTV promoter.

Requirement for the PRD Activation Domain of NF1 in MMTV Minichromosomes Activated in Vitro by PRb—To test whether the specific requirement for synergism between NF1 and GR was also found with another receptor and under different conditions, we used the same wild type and mutant MMTV promoters along with recombinant human PRb and NF1 for transcription experiments in vitro. To this end the corresponding templates were assembled into minichromosomes in extracts from Drosophila embryos and transcribed in a HeLa cell nuclear extract (22). As reported previously, the wild type MMTV promoter is organized into positioned nucleo-

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FIG. 5. Chromatin remodeling requires the DBD of NF1. Mapping of DHS was performed by indirect end labeling of transformants with plGZ2/MMTV (MMTV), p314N795 (GR), and either pAAH5 (empty vector), pCTF2 (NF1-C2), or pCTFb (NF1-C229). Cells were untreated or treated with DAC for 60 min as reported previously (30). The DHS over nucleosome B is indicated with white asterisks. The SacI (S) and DraI (D) cleavage sites used as internal markers are also indicated.

somess (32) and virtually repressed in these minichromosomes (22) (Fig. 6A, lane 1). Incubation with either PRB (lane 2) or NF1 (lane 3) resulted in very little transcription, whereas incubation with both proteins together led to very robust transcription (lane 5). In this particular experiment the synergism between PRB and NF1 was 13-fold. Recombinant NF1-DBD alone was unable to activate transcription on its own (lane 4), but in conjunction with PRB resulted in a similar transcriptional synergism as observed the intact NF1 protein (lane 6).

On minichromosomes containing the HRE/MMTVΔ promoter, the core promoter was more accessible to the basal transcriptional machinery than on wild type MMTV minichromosomes (compare lanes 1 and 7). PRB alone was able to activate transcription to a higher extent (compares lane 2 and 7 with lane 8), and NF1 also produced a slight increase in transcription (compare lanes 7 and 9). The two proteins did synergize on the HRE/MMTVΔ minichromosomes, although to a lesser extent than on wild type minichromosomes (synergism, 5-fold). The NF1-DBD was transcriptional inert on the HRE/MMTVΔ minichromosomes, although to a lesser extent than on wild type minichromosomes (synergism, 5-fold). The NF1-DBD was transcriptional inert on the HRE/MMTVΔ minichromosomes, although to a lesser extent than on wild type minichromosomes (synergism, 5-fold).

The accessibility for PRB of the HRE/MMTVΔ minichromosomes already indicates an open chromatin conformation. To test this notion in a more direct way we measured the cleavage efficiency at a HindII restriction site, which overlaps with the NF1 binding site and is located within the confines of nucleosome B (32). With wild type MMTV minichromosomes less than 2% of the templates were cleaved within 2 min (Fig. 6B, lane 3), suggesting a tight chromatin structure. In contrast, with HRE/MMTVΔ minichromosomes over 30% of the templates were cleaved during the same period of time (lane 4), indicating a more relaxed chromatin organization. The kinetics of cleavage of the two templates over a period of 20 min shows that this difference is stable over time and thus likely reflects a difference in the starting chromatin organization of the templates (Fig. 6B, inset).

DISCUSSION

The MMTV promoter is activated by glucocorticoids or progesterins in a process that involves a synergistic interaction between GR or PR and NF1. Based on titration experiments with wild type and mutant proteins in yeast, we have shown here that NF1 does not act like a conventional transcription factor in the context of the activated MMTV promoter. The PRD of NF1 is neither operative nor needed for synergism with GR in yeast. Following hormone induction and GR binding to the exposed HREs, binding of NF1 to its cognate site on the MMTV promoter is promoted, required, and sufficient for full activation of the GR-triggered promoter. Hence, there is a functional synergism between GR and the DBD of NF1. Similarly, on MMTV minichromosomes transcribed in vitro, PR synergizes with NF1 or its DBD, while the transcriptional domain is not required. This novel kind of synergism depends on the proper organization of the promoter in chromatin, which is mediated by the region containing the natural array of five HREs. Replacing the natural HREs by a single canonical HRE disturbs the chromatin organization and makes the synergism with GR or PR dependent on the PRD of NF1. Therefore, the lack of function of the PRD in the context of the MMTV promoter is not due to the inability of this domain to synergize
with GR in vitro or in yeast, nor to the absence of relevant co-activators in this organism.

In mammalian cells, hormone-dependent activation of the MMTV promoter is accompanied by the appearance of a DHS over the nucleosome B, which has been interpreted as the consequence of nucleosome disruption (16, 35). Following glucocorticoid induction of cells carrying chromosomally integrated copies of the MMTV promoter, we find an increase in nucleosome mobility in the vicinity of the pseudodyad axis of nucleosome B and a concomitant occupation of all the HREs and the NF1 site, as judged by genomic open footprinting (8). We interpreted these findings as indicative of an open nucleosome B conformation induced by GR binding and compatible with simultaneous binding of all transcription factors to the promoter. At that time we could not decide whether the activated receptor alone was able to induce this conformational change or whether NF1 was also needed. Our results in yeast now show that GR alone is not sufficient to induce a stable structural modification of chromatin. Rather NF1 is required for the generation of a DHS over nucleosome B. More importantly, the DBD of NF1 is sufficient for this effect. This suggests that the main function of NF1 in the context of the activated MMTV promoter is to bind to DNA and to stabilize an “open” chromatin conformation. The stabilizing effect may arise from the high affinity of NF1 for its target site on free DNA in combination with its inability to bind to this site on positioned nucleosomes containing a full complement of core histones (11, 14). The nature of the open chromatin conformation stabilized by NF1 binding remains obscure, but it may include a total or partial dissociation of histone H2A/H2B dimers, as NF1 binds efficiently to MMTV promoter sequences positioned on a tetramer of histones H3 and H4 (36).

Therefore, NF1 amplifies the transcriptional response of the MMTV promoter at low concentration of activated GR in vivo, likely by the same mechanism by which it reduces the amount of PR needed for full occupancy of the HREs in vitro (22). As shown in our titration experiments, this auxiliary role is accomplished with high efficiency, since very low amounts of NF1 are required to reach maximal activity. This mechanism of activation depends on the accurate chromatin organization of the MMTV promoter. Whereas, in response to the ligand the receptors can bind to well organized chromatin and thus initiate the activation of the promoter, NF1 is unable to trigger this process, because it cannot access its target site within positioned nucleosomes (11, 14). Therefore, NF1 can only act on promoters that have been fired by activated receptors. It cooperates with the receptors by maintaining an open chromatin structure, which is required for the entry of additional receptor molecules. The DBD of NF1 is sufficient for this structural role, which probably can only take place when the HREs and the NF1 binding sites are located within the same positioned nucleosome. Triggering by the receptors must be the rate-limiting step in the promoter activation cascade, as suggested by our observation that the levels of activated GR determines the absolute induction values. That this kind of synergism depends on correctly positioned nucleosomes is suggested by the results obtained when nucleosome positioning is disturbed, as in the case of the HRE/MMTVΔ promoter. This alteration in chromatin structure makes the NF1 site more accessible and enables NF1 to contribute to the activation of promoters via its own transactivation domain. In this situation, however, the DBD of NF1 alone is not sufficient. Although confirmation in animal cells is necessary, our results in yeast show that the precise chromatin organization over the regulatory region of MMTV allows a fine-tuning of the proviral promoter by providing a platform for factor cooperation.

Two models have been proposed to explain the activation of promoters by various inducing agents, the gradual model and the binary model. The gradual model assumes the existence of multiple states of promoter activity, depending on the dose of the inducing agent and the strength and combinations of transactivators. The stochastic binary model proposes that there are only two possible states of any promoter, “on” and “off,” and that the strength of a particular promoter is an intrinsic property of its core elements (37, 38). In this model, the increase in transcription levels found with increasing amounts of activators is attributed to the recruitment of an increasing proportion of promoters from the off into the on state. The binary model has been shown to apply to the hormonal induction of the MMTV promoter in mammalian cells (39) and may explain why the PRD of NF1 is not operative in the context of the wild type MMTV promoter.

It has been reported that a graded response can be converted into an all-or-none binary response by either a competition of transactivators and transrepressors for the same DNA regulatory element (40) or the introduction of a positive feedback loop (41). Our results are compatible with the participation of both mechanisms in the activation of the MMTV promoter. On the one side, a positioned nucleosome acts as a repressor that competes with binding of the transactivator NF1. On the other, receptor-mediated NF1 binding acts a positive feedback loop for binding of additional hormone receptors molecules. Along this line, we predict that the HRE/MMTVΔ will behave in a more graded fashion. Although our mechanistic view of the induction process remains purely hypothetical, it provides a theoretical frame for exploring how the same two transcription factors acting on the same core promoter synergize by two different mechanisms depending on the DNA sequence information context.

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REFERENCES

1. Peterson, C. L., and Tammk, J. W. (1995) Trends Biochem. Sci. 20, 143–146
2. Cairns, B. R. (1998) Trends Biochem. Sci. 23, 20–25
3. Kadonaga, J. T. (1998) Cell 92, 307–313
4. Imhof, A., and Wolffe, A. P. (1998) Curr. Biol. 8, R422–R424
5. Grunstein, M. (1997) Nature 389, 349–352
6. Beato, M., Truss, M., and Chavez, S. (1996) Ann. N. Y. Acad. Sci. 784, 93–123
7. Hager, G. L., Archer, T. K., Fraga, G., Brensick, E. H., Tsukagoshi, Y., John, S., and Smith, C. L. (1995) Cold Spring Harbor Symp. Quant. Biol. 58, 63–71
8. Truss, M., Bartsch, J., Schelbert, A., Hache, R. J. G., and Beato, M. (1995) EMBO J. 14, 1737–1751
9. Scheidereit, C., and Beato, M. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 3029–3033
10. Kruse, U., and Sippel, A. E. (1994) J. Mol. Biol. 238, 860–865
11. Eissfeldt, K., Candau, R., Truss, M., and Beato, M. (1997) Nucleic Acids Res. 25, 3733–3742
12. Kim, T. K., and Roeder, R. G. (1993) Trends Biochem. Sci. 18, 2086–2089
13. Mermod, N., O’Neill, E. A., Kelly, T. J., and Tjian, R. (1989) Cell 58, 741–753
14. Piña, B., Bruggemeier, U., and Beato, M. (1990) Cell 69, 719–731
15. Cordingly, M. G., Riegel, A. T., and Hager, G. L. (1987) Cell 48, 261–270
16. Zaret, K. S., and Yamamoto, K. R. (1984) Mol. Cell. Biol. 38, 29–38
17. Chalepakis, G., Arnesmann, J., Slatyer, E., Bruull, H. J., Gross, B., and Beato, M. (1988) Cell 53, 371–382
18. Mikkelsen, R., Borgeneyer, M., and Nowock, J. (1987) EMBO J. 6, 1355–1360
19. Bruggemeier, U., Rogge, L., Winnacker, E. L., and Beato, M. (1990) EMBO J. 9, 2233–2239
20. Kaifl, M., Gross, B., and Beato, M. (1990) Nature 344, 360–362
21. Chávez, S., and Beato, M. (1997) Proc. Natl. Acad. Sci. U.S.A. 94, 2885–2890
22. DiCroce, L., Koop, R., Venditti, P., Westphal, H. M., Nightingale, K. P., Corona, D. F., Becker, P. B., and Beato, M. (1999) Mol. Cell 4, 45–54
23. Chávez, S., Candau, R., Truss, M., and Beato, M. (1995) Mol. Cell. Biol. 15, 6987–6998
24. Sikorski, R. S., and Hieter, P. (1989) Genetics 122, 19–27
25. Adams, R., Gotschling, D., and Stearn, T. (1997) Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
26. Candau, R., Chávez, S., and Beato, M. (1996) J. Steroid Biochem. Mol. Biol. 57, 19–31
27. Yoshinaga, S. K., Peterson, C. L., Herskowitz, I., and Yamamoto, K. R. (1992) Science 258, 1598–1604
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28. Altmann, H., Wendler, W., and Winnacker, E. L. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3901–3905
29. Mumberg, D., Muller, R., and Funk, M. (1994) Nucleic Acids Res. 22, 5767–5768
30. Guarente, L. (1983) Methods Enzymol. 101, 181–191
31. McNabb, D. S., Xing, Y., and Guarente, L. (1995) Genes Dev. 9, 47–58
32. Venditti, P., Di Croce, L., Kauer, M., Blank, T., Becker, P. B., and Beato, M. (1998) Nucleic Acids Res. 26, 3657–3666
33. Knox, J. J., Rebstein, P. J., Manoukian, A., and Gronostajski, R. M. (1991) Mol. Cell. Biol. 11, 2946–2951
34. Meisterernst, M., Rogge, L., Foeckler, R., Karaghisoff, M., and Winnacker, E. L. (1989) Biochemistry 28, 8191–8200
35. Richard-Foy, H., and Hager, G. L. (1987) EMBO J. 6, 2321–2328
36. Spangenberg, C., Eisele, K., Stunz, W., Luger, K., Fuchs, A., Richmonds, T. J., Truss, M., and Beato, M. (1998) J. Mol. Biol. 278, 725–739
37. Walters, M. C., Flieger, S., Eidenmüller, J., Magis, W., Groudine, M., and Martin, D. I. K. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 7125–7129
38. Weintraub, H. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 5819–5823
39. Ko, M. S. H., Nakauchi, H., and Takahashi, N. (1990) EMBO J. 9, 2835–2842
40. Rossi, F. M., Krüger, A. M., Spicher, A., Groudine, M., and Blau, H. M. (2000) Mol. Cell. Biol. 6, 723–726
41. Becskei, A., Seraphin, B., and Serrano, L. (2001) EMBO J. 20, 2528–2535