Nuclear Factor 1 and Octamer Transcription Factor 1 Binding Preset the Chromatin Structure of the Mouse Mammary Tumor Virus Promoter for Hormone Induction*

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When the mouse mammary tumor virus (MMTV) is integrated into the genome of a mammalian cell, its long terminal repeat (LTR) harbors six specifically positioned nucleosomes. Transcription from the MMTV promoter is regulated by the glucocorticoid hormone via the glucocorticoid receptor (GR). The mechanism of the apparently constitutive nucleosome arrangement has remained unclear. Previous in vitro reconstitution of nucleosome(s) on small segments of the MMTV LTR suggested that the DNA sequence was decisive for the nucleosome arrangement. However, microinjection of MMTV LTR DNA in Xenopus oocytes rendered randomly distributed nucleosomes. This indicated that oocytes lack factor(s) that induces nucleosome positioning at the MMTV LTR in other cells. Here we demonstrate that specific and concomitant binding of nuclear factor 1 (NF1) and octamer factor 1 (Oct1) to their cognate sites within the MMTV promoter induce a partial nucleosome positioning that is an intermediary state between the randomly organized inactive promoter and the hormone and GR-activated promoter containing distinctly positioned nucleosomes. Oct1 and NF1 reciprocally facilitate each other’s binding to the MMTV LTR in vitro. The NF1 and Oct1 binding also facilitate hormone-dependent GR-DNA interaction and result in a faster and stronger hormone response. Since NF1 and Oct1 generate an intermediary state of nucleosome positioning and enhance the hormone-induced response, we refer to this as a preset chromatin structure. We propose that this state of NF1 and Oct1-induced chromatin presetting mimics the early step(s) of chromatin remodeling involved in tissue-specific gene expression.

Nucleosomes (i.e. the packaging unit of eukaryotic chromatin) are usually randomly organized on DNA in vivo, but there are particular DNA segments where they are translationally positioned (1, 2). Such a nucleosome arrangement is often found within gene regulatory regions during the stepwise differentiation process of tissue-specific genes (3–5). The specific pattern of nucleosome organization typically occurs only in the tissue(s) where the particular gene is to become active (3) or maintained permissive for induction (6). The translational nucleosome-positioning event is often correlated with the binding of trans-acting factors to their cognate DNA sites (3, 6). An alternative mechanism for maintaining a specific translational nucleosome positioning might be by the DNA sequence as such. Previous studies demonstrated that DNA sequence directs nucleosomal organization into a preferred rotational positioning in vivo (7). Furthermore, in vitro nucleosome reconstitution experiments demonstrated a strong rotational positioning directed by a artificial DNA-bending sequences (8, 9). However, such DNA-bending sequences did not confer detectable translational nucleosomal positioning in vivo (10, 11).

The MMTV LTR is known to harbor six translationally positioned nucleosomes, nucleosomes A–F, along its 1.2 kb of DNA (12–14). This specific chromatin organization was first reported to occur in tissue culture cells such as NIH3T3 cells (12) and in human breast cancer cells T47D (13). A similar translational nucleosome positioning was seen both in the absence and in the presence of hormone induction, albeit a distinct hormone-dependent DNase I-hypersensitive site, also revealed by hydroxyl radical footprinting analysis, was developed at the cluster of glucocorticoid receptor (GR) binding sites (i.e. the glucocorticoid response elements (GREs)) (12, 13, 15). A high resolution mapping of the translationally positioned nucleosomes of the inactive MMTV promoter showed a broad distribution of differently positioned nucleosomes (14) although with a preference around the locations previously obtained by low resolution mapping by indirect end labeling (12). The mechanism of this apparently constitutive translational positioning has remained unclear.

In vitro nucleosome reconstitution experiments with MMTV LTR DNA from the B-nucleosome region demonstrated a strongly preferred frame of rotational positioning (16, 17) that was similar to the rotational position seen in vivo (13, 18). The in vitro nucleosome reconstitution experiments also indicated a preferred translational nucleosome positioning similar to the organization seen in vivo, but they were conducted on rather small DNA segments (16, 17, 19, 20). In addition, in vitro chromatin reconstitution experiments of circular plasmid DNA harboring the MMTV LTR using Drosophila embryo extracts also suggested a preferred translational nucleosome positioning over the B-nucleosome region (21). Taken together, these studies suggested that the DNA sequence might be of impor...
The two ubiquitously expressed transcription factors nuclear factor 1 (NF1) (22) and the POU-homeodomain octamer-binding factor 1 (Oct1) (23) were previously shown to contribute to the transcriptional response of the MMTV promoter (24–28) via binding to their cognate response elements within the MMTV promoter (Fig. 1A). These conclusions were based on the introduction of mutations targeted to individual binding sites and/or their combinations and analysis by stable (26, 28) or transient transfection experiments in tissue culture cells (24, 27, 29). This revealed the importance of these two factors for basal as well as hormone-induced transcription at the MMTV promoter. Importantly, both NF1 (13, 25) and Oct1 (13, 30) were shown to be excluded from the inactive promoter and to gain access only in the hormone-activated state. These findings have led to the formulation of the "two-step" model as the mechanism for GR-activated MMTV transcription. It postulates that the nucleosomes in the MMTV LTR are constitutively positioned, possibly by virtue of the DNA sequence, and that this precise nucleosome positioning excludes binding of NF1, Oct1, and other basal transcription factors. The first part of the "two-step" induction is initiated by the hormone-activated GR, which binds sequence specifically to the GREs and recruits co-activator(s) and effectuates chromatin remodeling, resulting in an opened chromatin structure. The second step constitutes binding of NF1, Oct1, and other basal factors to the more accessible DNA, leading to induction of transcription (31, 32).

However, the reconstitution of the MMTV LTR chromatin structure by nuclear injection into Xenopus oocytes revealed a different picture. Here, the nucleosomes were randomly positioned in the absence of hormone, whereas the translational nucleosome positioning and the DNase I hypersensitivity at the GRE cluster were seen only after GR-mediated hormone induction (33). The lack of prepositioned nucleosomes in oocytes suggested that the constitutive translational nucleosome positioning seen in other cells is driven by factor(s) that are absent in oocytes. Here, the nucleosomes were randomly positioned to the inactive promoter (18). However, NF1 binding was weak in the absence of GR activation, and a 50-fold higher NF1 binding efficiency was seen in the hormone-induced state. We also showed that NF1 requires the co-expression of Oct1 in order to elicit a transcriptional effect (i.e., an increased basal and hormone-induced transcription from the MMTV promoter). Here we address the effect of these two factors on the MMTV chromatin structure in relation to their capacity to bind to DNA and to induce MMTV transcription in the absence and presence of hormone activation. NF1 and Oct1 reciprocally enhance each other’s DNA binding in vivo. Importantly, these two factors cooperate in mediating an intermediatory level of nucleosome rearrangements within the MMTV LTR as compared with that seen after GR induction (33). Furthermore, the hormone-induced GR binding and gene induction event is considerably faster in the presence of NF1 and Oct1. Hence, we refer to this structural state as a preset chromatin structure. We propose that the pre-setting of this retroviral enhancer/promoter plays a role in the virus life cycle and may be reminiscent of the stepwise and trans-acting factor-mediated chromatin pre-setting that occur during the establishment of tissue- and developmental-specific gene expression.

MATERIALS AND METHODS

Plasmids—The DNA construct referred to as the MMTV reporter DNA is pMMTV:M13, which harbors the 1.2-kb MMTV LTR fused to the herpes simplex thymidine kinase gene at position +137 of the MMTV promoter (33). The construction of the plasmids for in vitro production of mRNA for rat GR (33), pig NF1-C1 (here referred to as NF1), and human Oct 1 (18) have been described earlier. The pig NF1-C1:DBD, a C-terminal deletion of the NF1-C1 where amino acid residues 1–228 were maintained, thus ending with a threonine residue at the C terminus, was constructed for mRNA production. It was generated with a 0.5 μg concentration of 5′-GTCAGAATTCGAC-AGCCACATGTATTGCCCCGCTGCT-3′ and the C-terminal primer 5′-GTCAGCGCCGGGCTCAATGGAATCAGAAGC-3′. The PCR product and the RN3P vector DNA (34) were cut with EcoRI and NotI and purified with QiAquick (Qiagen) from SeaPlaque GTG agarose gel (BioWhitaker Molecular Applications, Rockland, ME) followed by ligation and plasmid preparation according to standard procedures. The construction of pMMTV:Octp:M13, where the −77/−63 NF1 site was deleted and an Xhol site was introduced without changing the promoter spacing, was made by a PCR with oligonucleotides 5′-GATCTCGAGCCAGACAGCTTATGTTAAGGTCTATGTA-3′ (coding strand) and 3′-GATCTCGAGTCATTGAAATCAGAAGAAG-3′ (noncoding strand) from the template pBSMTV:Tk-Xhol (i.e., the same MMTV:Tk construct as above but moved into the pBluescript® vector (Stratagene)). After trimming with Xhol and ligation, the construct thus obtained was transferred to M13 mp18 by restriction sites KpnI and Xbal and used for preparation of single-stranded DNA. The construction of pMMTV3OctdOctp:M13, where both Oct sites had been deleted, was performed in the same way using oligonucleotides 5′-GATCTCGAGTCGTGCACAATAATATAAAGGATGCTGA-3′ (coding strand) and 5′-GATCTCGAGCCAGATAGCCTATGAGGTC-3′ (noncoding strand).

Oocyte injections have been described previously (35). Hormone, triamcinolone acetonide (TA), was added to the oocyte medium at 1 μM concentration. GR mRNA was not injected in some of the oocyte pools that were not to be treated with hormone in order to avoid possible hormone contamination-mediated effects.

Quantification of Intranuclear Transcription Factors—Oocytes were injected with RNA coding for GR and Oct1 proteins and placed in oocyte medium, OR2, also containing [35S]methionine, 1000 Ci/mmol (Amersham Biosciences) at a concentration of 0.02 μCi/μl medium overnight. Then the nuclei were dissected and analyzed in pools of five by 12% SDS-PAGE (36). Relative amounts of GR and Oct1 were estimated by quantification on a Fuji Bio-Imaging analyzer BAS-2500 using the Image Gauge version 3.3.1. In agreement with the report of the nucleosome content in the respective proteins as previously described for calculation of intranuclear concentration of NF1 (18). An aliquot was also analyzed by immunoblotting with GR antiserum together with known amounts of GR purified from rat liver (16) to serve as a standard curve for calculation of absolute amounts of Oct1 that followed a linear correlation between injected RNA and translated protein. Based on these calculations done in different oocyte experiments with the same mRNA preparations and a similar time of translation, about 24 h, the intranuclear amounts of GR (after hormone treatment), NF1, and Oct1 were about 0.1, 0.01, and 0.06 pmol, respectively, in a typical injection experiment. Assuming an intranuclear volume of 40 nl, this will result in an intranuclear concentration of 2.5, 0.25, and 1.5 µM for GR, NF1, and Oct1, respectively.

Quantification of MMTV transcription by S1-nuclease has been described previously (18). All analyses were done in duplicate or triplicate. The oocyte homogenate was split into two aliquots, and then RNA was quantified in one half, and the amount of injected pMMTV:M13 reporter DNA in the other aliquot was quantified by primer extension as for the SacI accessibility assay (see below). Transcription was expressed as the ratio of MMTV RNA/MMTV DNA extracted from the oocyte homogenate.

Chromatin and Protein-DNA Interaction Analysis—The SacI accessibility assay, primer extension (33), DMS in vivo footprinting (35), and in situ cleavage by methidiumpropyl-EDTA Fe(II)/MPE (18) were done as described previously. Radioactivity scans and quantifications were carried out with a Fuji Bio-Imaging analyzer BAS-2500 using the Image Gauge version 3.3 software. Samples were routinely analyzed in duplicate in order to control for experimental variation.

RESULTS

NF1 and Dose-dependent Oct1 Stimulation of Hormone-activated MMTV Transcription—The MMTV promoter harbors a cluster of four GR-binding sequences (Fig. 1A, GRE I–IV, white boxes), one NF1-binding site (light gray box), and two octamer-binding sites (black boxes), one proximal (Oct p) and one distal site (Oct d) relative to the TATA sequence

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C. Astrand and O. Wrange, unpublished results.
The MMTV LTR of 1.2 kb harbors binding sites for various transcription factors. This was achieved by use of different hormone concentrations that control the level of intranuclear GR (35). Such experiments again showed a distinct stimulation of both basal and hormone-induced transcription in the presence of Oct1 and a further stimulation by NF1 and Oct1 co-expression, whereas NF1 in the absence of Oct1 did not produce any effect either at low or high GR concentration. Although the absolute levels of transcription both in the absence and the presence of hormone varied between experiments, there was a good reproducibility of the effect on transcription by these various factors in relative terms (Supplement 1). We conclude that the positive effect of concomitant expression of Oct1 and NF1 is maintained both at low or high GR concentration. Although the absolute levels of transcription both in the absence and the presence of hormone varied between experiments, there was a good reproducibility of the effect on transcription by these various factors in relative terms (Supplement 1). We conclude that the positive effect of concomitant expression of Oct1 and NF1 is maintained both at low or high GR concentration. Although the absolute levels of transcription both in the absence and the presence of hormone varied between experiments, there was a good reproducibility of the effect on transcription by these various factors in relative terms (Supplement 1). We conclude that the positive effect of concomitant expression of Oct1 and NF1 is maintained both at low or high GR concentration. Although the absolute levels of transcription both in the absence and the presence of hormone varied between experiments, there was a good reproducibility of the effect on transcription by these various factors in relative terms (Supplement 1). We conclude that the positive effect of concomitant expression of Oct1 and NF1 is maintained both at low or high GR concentration.
transcription factors also in the more distal DNA segments (38). Hence, it is possible that the NF1 and/or Oct1 effects detected here might be mediated by other, perhaps cryptic, sites within the MMTV LTR. We addressed this by construction of mutants deleting either the NF1 site or the two Oct1 sites (see “Materials and Methods”). Oocytes injected with wild type MMTV LTR or either of these two mutants alone or in combination with mRNA coding for NF1 and Oct1 revealed that the NF1- and Oct1-dependent increase in basal MMTV transcription was indeed dependent on both an intact NF1 site and intact Oct1 binding sites (data not shown). Hence, we conclude that the transcriptional stimulation of these two factors is mediated by their corresponding binding sites located within the MMTV promoter segment as illustrated in Fig. 1A. This is in agreement with previous results of mutational analysis performed in mammalian tissue culture cells (26, 27) and suggests that similar NF1- and Oct1-related mechanisms are operating in Xenopus oocytes when these factors are provided.

NF1 and Oct1 Cooperatively Induce a Nucleosome Re-arrangement in the MMTV LTR in the Absence of Hormone-activated GR—The chemical nuclease MPE was used to monitor the nucleosome organization in the MMTV promoter, since it preferably cleaves internucleosomal linker DNA and lacks sequence-specific DNA cutting activity (12, 13, 39). By first injecting the single-stranded MMTV DNA reporter and then the various combinations of GR, NF1, and Oct1 mRNAs into pools of Xenopus oocytes, we were able to monitor the effects of NF1 and Oct1 on the chromatin structure. Untreated oocytes showed a cleavage pattern of weak bands with an even smearlike background (Fig. 3, lanes 1 and 2). We previously utilized restriction enzyme mapping (33) and DNase I footprinting (18) to demonstrate that this represents a random translational nucleosome positioning but with a significant level of rotational positioning. In accordance with previous results (33), hormone treatment of GR mRNA-injected oocytes generated an active chromatin pattern of translationally positioned nucleosomes with the typical pattern of a hypersensitive segment over the B-nucleosome and protection over the C-nucleosome (Fig. 3, compare lanes 1 and 2 with lanes 3 and 4 and scans below). In the absence of hormone, the same random pattern was seen when either NF1 or Oct1 mRNA was injected in the absence of GR and hormone (lanes 5 and 6 and lanes 9 and 10) or when also GR mRNA was injected but hormone was not provided (33) (data not shown). Importantly, the injection of Oct1 mRNA together with NF1 mRNA caused a distinctly different chromatin pattern, thus indicating that a nucleosome rearrangement had been directed by the concomitant presence of these two factors. Importantly, this chromatin rearrangement occurred in the absence of hormone and GR (Fig. 3, see lanes 13 and 14, and scans). In the presence of hormone-activated GR, there was a similar pattern of hypercutting of the B-nucleosome and protection over the C-nucleosome as reported previously (lanes 15 and 16 and scans) (33, 35). Hence, an identical hormone-induced cleavage pattern was seen for all combinations of NF1 and Oct1 with the exception that the presence of NF1 also generated a protected area over the NF1-binding site (see lane 8, lane 16, and corresponding scans).

We then asked whether the NF1- and Oct1-induced chromatin rearrangement was dependent on ongoing transcription. This was relevant, since the Oct1 and NF1 also cooperate in increasing basal (i.e. hormone-independent) MMTV transcription (c.f. Fig. 2, A and B, or Fig. 4, top) and since we previously showed that the addition of hormone at a low concentration that generated only about 2% of maximal hormone-inducible MMTV transcription did induce a significant translational nucleosome positioning within the MMTV LTR (35). MPE in situ footprinting was performed in the absence or presence of hormone-activated GR, but in this case also in the presence or absence of α-amanitin, an inhibitor of RNA pol II transcription (40). S1-nuclease protection analysis confirmed that the transcription was abrogated by α-amanitin and that NF1 and Oct1 caused a distinct increase in basal MMTV transcription, but only in the absence of α-amanitin (Fig. 4, top). The presence of α-amanitin resulted in a lower level of overall MPE cleavage activity but did not have any effect on the digestion pattern (Fig. 4, see also the overlaid scans 10 + 14, lowest, for better comparison). This implies that the chromatin structure imposed at the MMTV LTR by NF1 and Oct1 did not depend on ongoing transcription. We previously showed that the hor-
mone-activated GR-induced nucleosome positioning was not dependent on MMTV transcription (33).

The NF1 and Oct1 dependent changes in the MPE cleavage pattern of the MMTV LTR are better resolved in Fig. 4 but are otherwise very similar or identical to the result shown in Fig. 3. The inactive promoter showed a weakly banded pattern over the entire LTR (Fig. 4, lanes 2 and 3 and scan of lane 2). This indicates a random translational nucleosome positioning; the banded pattern fits with the previously observed tendency of rotational nucleosome positioning (18). Hormone activation resulted in the typical cleavage pattern of positioned nucleosomes (Fig. 4, lanes 4 and 5 and scan of lane 4). Please note the hypercutting of the B-nucleosome and the linker segments flanking the D-nucleosome (see scans 4 and 12, black arrowheads) and the distinct protection over the C- and E-nucleosomes (scans 4 and 12, white arrowheads).

The oocytes containing both Oct1 and NF1, but lacking GR and hormone, rendered a more distinct MPE digestion pattern consisting of fewer but stronger bands as compared with the untreated oocytes (compare lanes 2 and 3 to lanes 10 and 11 and scans 2 and 10 and overlaid scans 2 + 10). A comparison of this NF1- and Oct1-induced pattern with the hormone-activated MPE cleavage pattern revealed several similarities. Hence, the hormone-dependent protection over the C- and E-nucleosome segments and the hypersensitive sites flanking the D-nucleosome were partly developed by the presence of NF1 and Oct1 in the absence of hormone (see scans 10, 4, and 12; compare protected and hypersensitive segments marked with white or black arrowheads, respectively; see also overlaid scans 10 + 12). This indicates that the NF1- and Oct1-induced chromatin arrangement represents an intermediate state between the random nucleosome positioning of the noninduced state and the fully developed translational nucleosome positioning induced by hormone activation. Interestingly, the B-nucleosome segment remained protected but was flanked by two hypersensitive sites at this intermediary chromatin state, thus arguing for translational positioning of the B-nucleosome (see lanes 10 and 11 and scan 10, black arrows). The proximal B-nucleosome segment also contained a distinctly protected area coinciding with the NF1 site (scan 10, white arrow). Hormone-activated GR together with NF1 and Oct1 developed a similar pattern as the activated GR alone with a strong hypercutting segment over the B-nucleosome and flanking the D-nucleosome and strong protection over the C- and E-nucleosome segments. The only distinct difference was the strongly protected DNA segment over the NF1 binding site (compare lanes 4 and 5 with lanes 12 and 13 and scans 4 and 12, white arrowhead at NF1 protection, and overlaid scans 4 + 12). That this protection indeed coincided with the NF1 site was confirmed by developing the MPE digestion pattern with primer extension and subsequent electrophoretic separation on a denaturing gel at base pair resolution (Supplement 2).

We conclude that NF1 and Oct1 together induce a rearrangement of the MMTV LTR chromatin that represents an intermediary state of chromatin structure between the inactive and the hormone-activated state.
Oct1 and NF1 Collaborate Reciprocally in Binding to the MMTV Promoter in the Presence and Absence of Hormone—

The cooperative effect of Oct1 and NF1 on the MMTV chromatin structure transition (Figs. 3 and 4) and on basal transcription (Figs. 2 and 4) (18) urged us to investigate the DNA-binding step. We have previously utilized DMS in vivo footprinting to show that GR and NF1 help one another to bind more efficiently to the MMTV promoter (18). As demonstrated by others (13), this method may also be used to monitor Oct1 binding. The potential influence of these three transacting factors on their respective DNA binding activity was addressed in pools of oocytes injected with the indicated combinations of GR, Oct1, and NF1 mRNA (Fig. 5A). Factor binding was monitored based on the protective effects from the DMS methylation of the N7 position at the indicated guanine(s), where these factors are known to cause protection by specific binding (Fig. 5A, white circles mark protected guanines for the indicated factor-binding sites (boxes to the left); these guanines are marked with black dots in Fig. 1A).

Hence, the specific binding at each DNA site could be quantified. This was achieved by PhosphorImager analysis of two independently DMS-treated samples for each combination of factors in the absence and presence of hormone (TA).

As previously shown, the methylation protection at the GREs was completely hormone-dependent (Fig. 5A, GRE diagram; black bars, no hormone); in fact, the GR protein is not detectable within the oocyte nucleus in the absence of hormone (35, 36). The presence of NF1 stimulated GR binding (Fig. 5A, GRE diagram; compare lanes 3 and 4, showing 46% methylation, with lanes 11 and 12 showing 41% methylation). Importantly, there was a further increase in GRE methylation protection in the presence of both NF1 and Oct1 (GRE diagram, lanes 15 and 16, with 32% methylation). Evidence that the NF1 and Oct1-mediated stimulation of hormone-dependent GR binding is indeed significant is presented in Fig. 5B (Student’s t test, p = 0.002), lanes 13–15 versus lanes 16–18, in the GRE diagram). Interestingly, Oct1 alone did not enhance GR binding (Fig. 5A, GRE diagram, compare lanes 3 and 4 with lanes 7 and 8).

Methylation protection at the NF1 DNA site was detectable in all oocytes where NF1 mRNA had been injected (Fig. 5A, NF1 diagram, lanes 9–16), and, as shown previously (18), there was distinct NF1 binding in the absence of hormone (compare lanes 1–8, showing ~100% methylation with lanes 9 and 10, showing 89% methylation) and a dramatic hormone-dependent increase in NF1 methylation protection (NF1 diagram, compare lanes 9 and 10, showing 89% methylation, with lanes 11 and 12, showing 33% methylation). Importantly, the concomitant presence of NF1 and Oct1 resulted in an increased NF1 methylation protection both in the absence, from 89 to 83%, and in the presence of hormone-activated GR, from 33 to 19% (NF1 diagram compare lanes 9 and 10 with lanes 13 and 14 and lanes 11 and 12 with lanes 15 and 16). Evidence that an increasing amount of NF1 and Oct1 is correlated with increased specific NF1 binding in the absence of hormone and GR is presented in Fig. 5B (NF1 diagram, Student’s t test, p < 0.001, lanes 1–3 versus lanes 7–9). We conclude that Oct1 helps NF1 to bind its DNA site both in the absence and in the presence of hormone.

Oct1 binding was monitored both at the distal (Oct d) and the proximal (Oct p) binding site in the MMTV promoter (Fig. 5A, Oct diagrams). The DMS methylation protection by Oct1 was weaker than that obtained with GR and NF1, although similar amounts of Oct1 and GR were expressed in the oocytes (Fig. 1B and data not shown). However, there was a distinct GR- and hormone-dependent stimulation of Oct1 methylation protection at both sites (Fig. 5A, Oct d and Oct p diagrams, compare lanes 5 and 6 with lanes 7 and 8). Oct d was more protected than Oct p in the hormone-activated promoter (Fig. 5, A and B). Evidence that increasing amounts of NF1 and Oct1 correlate with significant constitutive Oct1 binding to the distal and the proximal Oct site is presented (Fig. 5B, Student’s t test, p = 0.005 and p < 0.001 for the highest Oct1 amount; i.e. lanes 10–12 versus lanes 1–3, for Oct d and Oct p, respectively). The concomitant expression of Oct1 and NF1 enhanced Oct1 methylation protection at the Oct sites both in the absence (Fig. 5A, compare lanes 5 and 6 showing 103% methylation with lanes 13 and 14 showing 92% methylation) and in the presence of hormone (Oct d diagram, compare lanes 7 and 8 showing 74% methylation with lanes 15 and 16 showing 63% methylation). We conclude that in the presence of NF1 there is a low level of Oct1 that is constitutively bound to the MMTV promoter also in the absence of hormone and GR. As opposed to NF1 that showed significant DNA binding when present alone, we were not able to detect any constitutive Oct1 binding in the absence of NF1 and hormone. However, we note that Oct1 methylation protection is weak, and hence the analysis of this DNA binder is less sensitive.

Furthermore, as opposed to the GREs and the NF1 site that lacked any sign of endogenous DMS methylation protection activity, there was a significant hormone-dependent protection at both Oct1 sites also in the absence of injected Oct1 mRNA (Fig. 5B, lanes 13–15, 93 ± 2.7%, p = 0.05 and 94 ± 3.2%, p = 0.04 for Oct d and Oct p, respectively). This suggests that a low level of endogenous Oct-binding activity is present in the oocytes. This may be in agreement with previous reports that described octamer-binding POU-domain proteins in Xenopus oocytes (41, 42). Importantly, our results show that these putative endogenous Oct-binding proteins are present in only trace amounts relative to injected DNA templates (Fig. 5, A and B) and that these endogenous proteins do not cooperate significantly with NF1 in eliciting a transcriptional response (Fig. 2B) (18) or in chromatin presetting (Fig. 3).

From these data and another two independent experiments, we conclude that both GR and NF1 stimulate Oct1 binding and that Oct1 and NF1 reciprocally facilitate the DNA binding of one another to their adjacent DNA sites in the absence of hormone. The reciprocally facilitated NF1 and Oct1 binding in the absence of hormone was weak but corroborates the Oct1 and NF1 stimulatory effect on transcription (Figs. 2 and 4 (top)). The titration of increasing amounts of injected mRNA for NF1 and Oct1 (Fig. 5B) also correlated with increasing levels of basal MMTV transcription (data not shown). The reciprocally facilitated DNA binding of NF1 and Oct1 is in excellent agreement with the effect of these factors on the MMTV chromatin structure (Fig. 3).

The 505-amino acid porcine NF1-C1 protein contains a DNA binding and dimerization domain within the N-terminal half and a proline-rich activation domain in the C-terminal region (22). We deleted the C-terminal part to leave the first 228 amino acids intact, which maintained the DNA binding and dimerization domain (NF1-DBD) (22, 43). This truncated protein was evaluated for collaborative capacity with Oct1 in terms of transcription and DNA binding by DMS in vivo footprinting as above. Two independent experiments did not reveal any functional difference when comparing this truncated protein with the wild type NF1-C1, either in terms of Oct1 cooperative effect on MMTV transcription or on Oct1 DNA binding by DMS in vivo footprinting (data not shown).

NF1 and Oct1 Generate a Faster Hormone Response in Terms of GRE Binding, Chromatin Remodeling, and MMTV Transcription—Since the Oct1- and NF1-induced chromatin rearrangement was reminiscent of a partially hormone- and GR-activated chromatin structure, it posed the question of whether
FIG. 5. GR, NF1, and Oct1 facilitate each other’s binding to the hormone-activated promoter (A), and NF1 and Oct1 bind constitutively to its cognate sites in the MMTV promoter in the absence of GR and hormone (B). A, DMS methylation protection analysis of oocytes injected with 3 ng of single-stranded MMTV reporter. 4 h later, they were injected with different mRNA mixes containing the indicated combinations of GR, NF1, and Oct1 mRNA (3.8, 0.5, and 6.3 ng, respectively) per oocyte. Hormone (TA) was added to the indicated oocytes 22 h after DNA injection, and then DMS analysis was done 11 h later. 2 × 10 oocytes were analyzed for each factor combination and analyzed individually and presented as duplicated bars to illustrate the reproducibility of the method. Methylation protection of indicated bands (white circles) was quantified and related to the sum of indicated reference bands (black dots) setting the GR-TA data (black staples in GRE III/H11003 VI diagram) to 100% methylation for each combination of injected transactive factor RNA. In the NF1 diagram, samples lacking NF1 mRNA (lanes 1–8) were set to 100% on the average. In the Oct diagrams, the average of lanes 1 and 2 and lanes 9 and 10 was set to 100%. The different factor binding sites are illustrated by boxes (left). The methylation protection (percentage) for each sample is presented in the diagrams for the indicated binding sites. The average methylation protection (percentage) is given above each pair of bars. B, oocytes injected with 1.5 ng of single-stranded MMTV reporter DNA as above and with increasing amounts of mRNA coding for NF1 (0.4, 0.8, and 1.6 ng) and Oct1 (2, 4, and 8 ng) or GR (3.8 ng) or GR and NF1 and Oct1 (3.8, 0.8, and 4 ng), respectively. 3 × 5 oocytes where injected and analyzed as three individual samples; results in diagrams are average of triplicates, with error bars signifying the S.D.
NF1 and Oct1 Preset Chromatin in MMTV LTR

Fig. 6. NF1 and Oct1 generate a faster and more efficient hormone response in terms of GR binding, chromatin remodeling, and MMTV transcription than GR alone. A, mRNA coding for the indicated proteins in amounts as in the legend of Fig. 5A were injected in oocytes, followed by 3 ng of single-stranded MMTV DNA reporter. 14 h after the DNA injection, hormone was added at different time points as indicated. B, DMS in vitro footprinting analysis of GR-GRE binding was plotted as a function of time (h). Oocytes treated the same way (see above) were analyzed for chromatin remodeling by the SacI accessibility assay and developed by primer extension (C) and were analyzed for MMTV transcription by S1-nuclease protection (D). Double samples were analyzed for each time point (see diagrams in B, C, and D).

this apparent transition state might influence the kinetics of the hormone response. This was addressed by the addition of hormone at different times to pools of oocytes injected either with GR mRNA alone or together with NF1 and Oct1 mRNAs (Fig. 6A). The comparison of the GR-DNA binding by DMS in vitro footprinting revealed significantly faster GR-DNA binding in the presence of NF1 and Oct1 than that obtained with GR alone (Fig. 6B). Specifically, there was 86% DMS methylation at the GREs for the GR only, as compared with 59% for the GR-, NF1-, and Oct1 mRNA-injected oocytes after 1 h of hormone incubation. Hence, the presence of NF1 and Oct1 resulted in a 3-fold higher GR binding in the Oct1- and NF1-containing oocytes at this early time point. This large difference in GR-GRE binding was reduced with increasing time of hormone incubation (Fig. 6B).

Chromatin remodeling, as quantified by SacI in situ cutting, also showed faster hormone induction kinetics for GR-, NF1-, and Oct1 mRNA-injected versus GR-injected oocytes (Fig. 6C). The two curves converge at 4 h, showing that NF1 and Oct1 increased the speed of GR-induced chromatin remodeling but did not alter its final level. The slightly lower SacI cutting in the Oct1- and NF1-containing oocytes before hormone addition (Fig. 6C, time 0 h) was a reproducible finding showing that the local chromatin structure around the SacI site is less accessible in this context.

The MMTV transcription also showed an increased rate of induction for the GR-, NF1-, and Oct1 mRNA-injected oocytes (Fig. 6D). Both chromatin remodeling and transcription showed the strongest difference between the two groups of oocytes at 2 h (Fig. 6, C and D), whereas the maximal difference in GR-DNA binding occurred at 1 h after hormone addition (Fig. 6B). This agrees with GR-DNA binding being the primary event, which then leads to chromatin remodeling and MMTV transcription. Similar results were obtained in three independent experiments. From this, we conclude that the NF1- and Oct1-rearranged chromatin provide a more rapid GR-GRE binding reaction that in turn results in a faster chromatin remodeling and transcriptional response.

DISCUSSION

Our results demonstrate for the first time that the constitutive binding of the ubiquitous factors NF1 and Oct1 to the MMTV promoter alters its chromatin structure from randomly positioned nucleosomes to a partial translational positioning. This chromatin remodeling represents an intermediate state between noninduced randomly positioned nucleosomes and the hormone-induced chromatin structure that involves a distinct nucleosome positioning (Fig. 4). Our results argue against a primary role for DNA sequence in setting up the specific chromatin structure but do not exclude the possibility that the DNA sequence is directing the nucleosomes to termodynamically preferred positions once the process has been initiated by NF1 and Oct1 binding. The trans-acting factor-induced nucleosome positioning of the MMTV LTR is reminiscent of, for example, the albumin enhancer, where tissue specifically expressed trans-acting factors were shown to play a major role in causing a translational nucleosome positioning (3). The NF1- and Oct1-induced chromatin structure also correlates with an increased basal transcription, a faster GR-GRE binding, and a faster and more efficient hormone response. Taken together, this motivates the term “preset chromatin” to be used for the NF1- and Oct1-induced chromatin state.

NF1 and Oct1 Trigger a Dynamic Chromatin Transition in the MMTV LTR—NF1 binding was barely detectable by DMS in vivo footprinting when expressed in the absence of the hormone-activated GR, and Oct1 did not have any detectable constitutive binding (Fig. 5A). However, there was a significant level of binding when both proteins were expressed simultaneously (Fig. 5, A and B), showing that they collaborate reciprocally in achieving specific DNA binding at the NF1 site and at the Oct sites. A previous report based on in vitro DNase I footprinting demonstrated a nonreciprocal co-operation in the MMTV promoter, where NF1 binding stabilized Oct1 binding (28). Our results show that both proteins are required to generate the chromatin rearrangement (Fig. 3) and that they cooperatively increase the basal transcription (see Fig. 2, Supplement 1, Fig. 4, and Ref. 18). This is corroborated by our in vivo footprinting data showing that both proteins facilitate each other’s binding (Fig. 5, A and B). We recently reported that NF1 alone is able to bind its cognate site in the MMTV promoter in the inactive promoter in vivo, albeit with a low affinity/accessibility, and that a −50-fold increased NF1 site accessibility is achieved by hormone activation (18). Here we show that a similar GR-mediated stimulation of Oct1 binding is seen upon
hormone activation (Fig. 5, A and B). The same finding was reported previously (13, 25), although these authors did not detect any constitutive binding of NF1 and Oct1. However, only barely detectable levels of hormone-independent binding of NF1 and Oct1 were seen also in our system (Fig. 5B). It is likely that such a low level of binding was not detectable in the previous studies using much lower copy number systems (13, 25). The GR-mediated stabilization of NF1 and Oct1 binding (Fig. 5, A and B) may be due to the chromatin remodeling mediated by GR, presumably via recruitment of various nucleosome remodeling/coactivator complexes (44–46). Alternatively, it may be due to a direct protein-protein contact between the GR DNA binding domain and Oct1, as has been reported before (30) and/or complex formation between NF1 and Oct1, as recently described to occur between NF1 and the homeobox containing thyroid transcription factor 1 (47). These different mechanisms may be operating concomitantly. Also, the hormone-activated GR-GRE binding is stimulated by concomitant binding of NF1 and Oct1 (Fig. 5, A and B). This is in good agreement with the cooperation of these factors concerning the transcriptional response (see Fig. 2, Supplement 1, and Ref. 18). We hypothesize that the mechanism of NF1- and Oct1-mediated stimulation of transcription may be due to a stabilized enhancerosome complex, thus offering a more stable platform for the assembly of the preinitiation complex (c.f. Ref. 18) (data not shown).

Others have reported on a weak binding of NF1 in the context of preset chromatin. This concerned the stepwise chromatin organization of the enhancer segments of the lysozyme gene during myeloid differentiation (5, 6). Interestingly, no NF1 binding was detected by DMS in vivo footprinting in the cell line corresponding to the multipotent progenitor cells. However, DMS methylation protection of the NF1 sites was detectable at the next step of differentiation corresponding to resting macrophages. Intriguingly, when applying the method of chromatin immunoprecipitation, they found that NF1 was weakly associated with the target enhancer segments already in the multipotent progenitor cells, whereas NF1 binding was not seen in the erythroblast that lack positioned nucleosomes in the lysozyme enhancers. Hence, the binding of NF1 was correlated with the appearance of preset chromatin at the lysozyme gene enhancers (6). We find these results to be reminiscent of our results concerning the presetting of the MMTV LTR by NF1 and Oct1, and we hypothesize that this exemplifies a chromatin transition state that is generally involved in setting up tissue-specific gene expression.

The weak NF1 and Oct1 binding during chromatin presetting (Fig. 5, A and B) argues for a chromatin-mediated restriction in accessibility of their target sites that generates a dynamic equilibrium in which NF1 and Oct1 interact with the majority of MMTV templates over time but where only a small fraction is bound at each instant. That the vast majority of MMTV templates in the oocyte nuclei are indeed engaged in the chromatin presetting is evident from the distinct changes in the MPE footprinting pattern (Figs. 3 and 4) and by the faster kinetics of GR binding and chromatin remodeling induced by NF1 and Oct1 (Fig. 6). NF1 is known to have a high DNA binding affinity (48) that would be expected to exceed the local histone-DNA affinity once NF1 has been allowed to bind, and our data suggest that this affinity is even higher in the presence of Oct1 (Fig. 5A). It is thus astonishing that the rather weak but significant DMS footprint of NF1 and Oct1, which can be seen more distinctly by injecting more NF1 and Oct1 mRNA (Fig. 5B), remains at such a low level in the absence of hormone-activated GR. We speculate that a transient and dynamic nature of both NF1 and Oct1 binding during this chromatin presetting stage is caused by a rapid fluctuation between an accessible state and an inaccessible state of their cognate binding sites. These fluctuations of the binding targets would thus be able to disrupt previously formed protein-DNA complexes and render a short residence time for NF1 and Oct1 binding to their cognate DNA sites. This may be reminiscent of the hit-and-run mechanism that has been suggested to operate with the help of SWI/SNF remodeling during GR binding and release (49).

The NF1- and Oct1-induced chromatin presetting occurs also in the presence of α-amanitin, which inhibits RNA polymerase II-driven transcription (Fig. 4). This suggests that NF1 and Oct1 binding to DNA is directly involved in mediating the change of the chromatin structure (Fig. 7). Determination of whether this also involves the recruitment of chromatin remodeling/modification complex(es) and histone tail modifications awaits further analysis. We were surprised to find that the deletion of the NF1 C-terminal half that contains the proline-rich activation domain did not have any different effect than the wild-type NF1. However, it corroborates the recently reported in vivo binding of the NF1-DBD with the homeobox-containing thyroid transcription factor 1 (47).
Possible Function of a Preset Chromatin Structure in Viral Propagation and Cellular Differentiation—NF1-C1 (22) and Oct1 (23) are members of two separate gene families of transcription factors that both consist of several different genes that are alternatively spliced. Family members and splice variants of both classes are known to be tissue-specifically expressed in many different cell types and to collaborate with many other DNA-binding proteins in gene regulation. Furthermore, both these families use the same or similar DNA binding sites among the family members, albeit specific for the NF1 and the Oct family, respectively. A retrovirus such as the MMTV needs to be inserted into the host genome and then expressed in order to produce progeny. The integrated provirus must cope with the repressive effects of the host chromatin structure and its chromatin-mediated silencing mechanisms (50). Furthermore, the MMTV retrovirus may have to infect many different cell types before it reaches the mammary gland, where it needs to be located to infect the next generation of mice, presumably via the milk (51). The MMTV is known to have a broad tissue specificity in vivo (52), and it is likely that the NF1 and Oct DNA sites in the MMTV promoter contribute to this. Chromatin-mediated silencing seems to be counteracted by ongoing transcription (53). This implies that the Oct1- and NF1-mediated increase in basal MMTV transcription and/or the preset chromatin structure is of importance to countercr chromatin silencing and thereby maintain the potential for hormone-mediated gene induction. It is a common theme during early tissue-specific differentiation that the transcription of a specific gene is turned on at a low level during the early stages of the chromatin remodeling process (6) or that intergenic transcription is involved in chromatin activation of an inactive locus (54). Thus, we propose that the MMTV virus utilizes the ubiquitously expressed NF1 and Oct family members to exploit the same pathway that is used in metazoans to set up tissue-specific gene patterns.

The Mechanism of Nucleosome Positioning of the MMTV LTR: Interactive Effects of Transcription Factors on Chromatin Structure—Our finding of the appearance of an intermediary state of translational nucleosome positioning upon concomitant expression of NF1 and Oct1 argues strongly that the binding of these trans-acting factors is the direct cause of the hormone-independent nucleosome positioning of the MMTV LTR and suggest that the same or similar factors maintained this chromatin structure that was previously observed in other cells (12, 13). That the ubiquitously expressed NF1 and Oct1 were indeed present in the cells of these previous experiments was demonstrated by various footprinting experiments (13, 25, 26). The fact that the MMTV LTR harbors randomly positioned nucleosomes when introduced into Xenopus oocytes (33) is thus explained by the lack of NF1 and Oct1 in oocytes, at least in relative terms.

It should be added that other factors that differ between the Xenopus oocyte system and mammalian cells may have an impact on the tissue-specific gene regulation by nuclear receptors. For example, Xenopus oocytes lack histone H1 and contain the more weakly bound embryonic linker histone B4 instead (55). Furthermore, the content of various histone-modifying complexes, nuclear receptor recruited coactivators, corepressors, and chromatin remodeling complexes (56) may have a tissue-specific distribution. The fact that the hormone-dependent GR activation of the MMTV promoter and the nucleosome structure of the MMTV LTR thus obtained is similar between oocytes and mammalian cells does, however, indicate that the oocyte system is useful for mechanistic studies.

Previous work suggested that the nucleosome positioning is important for restricting the access of NF1, Oct1, and other basal factors to their cognate binding sites, as shown in vitro for NF1 (17, 57, 58). During hormone induction, it was assumed that the hormone-activated GR binds and mediates a chromatin-remodeling event that will then allow binding of NF1, Oct1, and other basal factors to the MMTV promoter. This is usually referred to as the two-step model (25, 32, 59). Two important differences from the two-step model follow from our results: (i) NF1 and Oct1 interact, albeit at low level, with their cognate binding sites without any support from GR-driven remodeling, and (ii) NF1- and Oct-mediated chromatin presetting assists GR binding and renders a faster hormone response. Once GR is bound, it will greatly enhance NF1 and Oct1 binding (Fig. 7), presumably by chromatin opening via recruitment of nucleosome remodeling complexes (44, 45). Hence, our results imply a more interactive cooperation between the DNA binding factors and chromatin, leading to a stepwise development of more activity-prone chromatin states with an ultimate end point of a transcriptional response.

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REFERENCES

1. Simpson, R. T. (1991) Prog. Nucleic Acids Res. Mol. Biol. 40, 143–184
2. Wallrath, L. L., Lu, Q., Granok, H., and Elgin, S. C. (1994) BioEssays 16, 165–170
3. McPherson, C. E., Shim, E.-Y., Friedman, D. S., and Zaret, K. S. (1993) Cell 75, 387–398
4. Gualdi, R., Bossard, P., Zheng, M., Hamada, Y., Coleman, J. R., and Zaret, K. S. (1996) Genes Dev. 10, 1670–1682
5. Huber, M. C., Kruger, G., and Bonifer, C. (1996) Nucleic Acids Res. 24, 1443–1452
6. Lefevre, P., Melnik, S., Wilson, N., Riggs, A. D., and Bonifer, C. (2003) Mol. Cell. Biol. 23, 4348–4357
7. Drew, H. R., and Travers, A. A. (1985) J. Mol. Biol. 186, 773–790
8. Shrader, T. E., and Crothers, D. M. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 7418–7422
9. Li, Q., and Wrange, O. (1995) Mol. Cell. Biol. 15, 4375–4384
10. Lu, Q., Wallrath, L. L., and Elgin, S. C. (1995) EMBO J. 14, 4738–4746
11. Tanaka, S., Zatchej, M., and Thoma, F. (1992) EMBO J. 11, 1187–1193
12. Vendetti, P., Di Croce, L., Kauer, M., Blank, T., Becker, P. B., and Beato, M. (1999) Cell 96, 719–731
13. Truss, M., Bartsch, J., Schulbert, A., Hache, R. J. G., and Beato, M. (1995) EMBO J. 14, 1737–1751
14. Forrester, G., John, S., Roberts, M. S., and Hager, G. L. (1995) Gene 1933, 1947
15. Zaret, K. S., and Yamamoto, K. R. (1984) Cell 38, 29–38
16. 17. Pina, B., Bruggemeier, U., and Hager, G. L. (1990) EMBO J. 9, 2781–2787
18. Belikov, S., Astrand, C., Holmqvist, P. H., and Wrange, O. (2004) Mol. Cell. Biol. 24, 3036–3047
19. Almouzni, G., and Wolffe, A. P. (1993) Nature 361, 1573–1576
20. Spangenberg, C., Riesfeld, K., Kunkel, W., Kluh, F., Als, A., Richmond, T. J., Truss, M., and Beato, M. (1999) J. Mol. Biol. 278, 725–739
21. Vendetti, P., Di Croce, L., Kauer, M., Blank, T., Becker, P. B., and Beato, M. (1999) Nucleic Acids Res. 26, 3657–3666
22. Gronostajski, R. M. (2000) BioEssays 22, 31–45
23. Phillips, K., and Luisi, B. (2000) J. Mol. Biol. 302, 1023–1039
24. Buetti, E., and Kuhnbel, B. (1986) J. Mol. Biol. 190, 379–389
25. Cordingly, M. G., Riegel, A. T., and Hager, G. L. (1987) Cell 48, 261–270
26. Buetti, E. (1994) Mol. Cell. Biol. 14, 1191–1203
27. Toohey, M. G., Lee, J. W., Huang, M., and Peterson, D. O. (1990) J. Virol. 64, 4477–4484
28. Buetti, E., and Kuhnbel, B. (1986) J. Mol. Biol. 190, 379–389
29. Cato, A. C., Skroch, P., Weinmann, J., Butkeraitis, P., and Ponta, H. (1988) EMBO J. 7, 1403–1410
30. Pina, B., Bruggemeier, U., and Beato, M. (1999) Cell 96, 719–731
31. Cattoglio, G., and Wolffe, A. P. (1993) Genes Dev. 7, 2033–2047
32. Mink, S., Hartig, E., Jennewein, P., Doppler, W., and Cato, A. C. (1992) Mol. Cell. Biol. 12, 4906–4918
33. Cartwright, I. L., Hertzigberg, R. P., Dervan, P. B., and Elgin, S. C. (1983) Proc.
NF1 and Oct1 Preset Chromatin in MMTV LTR

40. Lindell, T. J., Weinberg, F., Morris, P. W., Roeder, R. G., and Rutter, W. J. (1970) Science 170, 447–449
41. Whitfield, T., Heasman, J., and Wylie, C. (1993) Dev. Biol. 155, 361–370
42. Hinkley, C. S., Martin, J. F., Leibham, D., and Perry, M. (1992) Mol. Cell. Biol. 12, 638–649
43. Prado, F., Koop, R., and Beato, M. (2002) J. Biol. Chem. 277, 4911–4917
44. Ostlund Farrants, A.-K., Blomquist, P., Kwon, H., and Wrange, O. (1997) Mol. Cell. Biol. 17, 895–905
45. Fryer, C. J., and Archer, T. K. (1998) Nature 393, 88–91
46. Kadam, S., and Emerson, B. M. (2003) Mol. Cell 11, 377–389
47. Bachurski, C. J., Yang, G. H., Currier, T. A., Gronostajski, R. M., and Hong, D. (2003) Mol. Cell. Biol. 23, 9614–9624
48. Roulet, E., Bucher, P., Schneider, R., Wingender, E., Dusserre, Y., Werner, T., and Mermod, N. (2000) J. Mol. Biol. 297, 833–848
49. Nagaich, A. K., Walker, D. A., Wolford, R., and Hager, G. L. (2004) Mol. Cell 14, 163–174
50. Grewal, S. I., and Moazed, D. (2003) Science 301, 798–802
51. Gunzburg, W. H., and Salmons, B. (1992) Biochem. J. 283, 625–632
52. Hennard, D., and Ross, S. R. (1988) J. Virol. 62, 3046–3049
53. Zink, D., and Paro, R. (1995) EMBO J. 14, 5660–5671
54. Fu, X. H., Liu, D. P., and Liang, C. C. (2002) Exp. Cell Res. 278, 1–11
55. Ura, K., Nightingale, K., and Wolffe, A. P. (1996) EMBO J. 15, 4949–4969
56. Wang, W., Côte, J., Xue, Y., Zhou, S., Khavari, P. A., Bigger, S. R., Muchardt, C., Kalpana, G. V., Goff, S. P., Yaniv, M., Workman, J. L., and Crabtree, G. R. (1996) EMBO J. 15, 5379–5382
57. Archer, T. K., Cordingley, M. G., Wolford, R. G., and Hager, G. L. (1991) Mol. Cell. Biol. 11, 688–698
58. Blomquist, P., Li, Q., and Wrange, O. (1996) J. Biol. Chem. 271, 153–159
59. Beato, M., and Eisfeld, K. (1997) Nucleic Acids Res. 25, 3559–3563