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Constitutive Repression and Nuclear Factor I-Dependent Hormone Activation of the Mouse Mammary Tumor Virus Promoter in *Saccharomyces cerevisiae*

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To study the influence of various transactivators and the role of nucleosomal structure in gene regulation by steroid hormones, we have introduced mouse mammary tumor virus (MMTV) promoter sequences along with expression vectors for the glucocorticoid receptor (GR) and nuclear factor I (NFI) in *Saccharomyces cerevisiae*, an organism amenable to genetic manipulation. Both in the context of an episomal multicopy vector and in a centromeric single-copy plasmid, the MMTV promoter was virtually silent in the absence of inducer, even in yeast strains expressing GR and NFI. Induction was optimal with deacylcortivazol and required both GR and NFI. The transactivation function AF1 in the N-terminal half of GR is required for ligand-dependent induction and acts constitutively in truncated GR lacking the ligand binding domain. A piece of the MMTV long terminal repeat extending from −236 to +111 is sufficient to position a nucleosome, B, over the regulatory region of the promoter from −45 to −190 and another nucleosome over the transcription start region. The rotational orientation of the DNA on the surface of nucleosome B is the same as that previously found in animal cells and in reconstitution experiments. This orientation is compatible with binding of GR to two sites, while it should preclude binding of NFI and hence be responsible for constitutive repression. Upon ligand induction, there is no major chromatin rearrangement, but the proximal linker DNA, including the TATA box, becomes hypersensitive to nucleases. The transcriptional behavior of the MMTV promoter was unaffected by deletions of the genes for zuotin or SIN1/SPT2, two proteins which have been claimed to assume some of the functions of linker histones. Thus, despite the lack of histone H1, yeast cells could be a suitable system to study the contribution of nucleosomal organization to the regulated expression of the MMTV promoter.

Regulation of gene expression in animal cells is achieved by a complex combinatorial interplay of constitutive and modulable transcription factors. Transcription factors can be grouped into large heterogeneous families, with each particular cell type containing a complex subset of each family member. Within a given family of factors, the functions of individual members differ only in subtle ways, and this hampers the interpretation of transfection results in animal cells. One way of simplifying the analysis is to reconstitute the regulatory system in more simple organisms, like the yeast *Saccharomyces cerevisiae*, provided they are deprived of the corresponding transcription factors. By deliberately expressing in yeast cells a particular subset of factors, one can study their function without interference by related members of the gene family. We have tried to use this approach to study the molecular interactions mediating hormonal regulation of the mouse mammary tumor virus (MMTV) promoter.

The MMTV promoter is subjected to a tight regulation in most animal cells. The promoter is virtually silent in the absence of steroid hormones and becomes activated after addition of glucocorticoids or progestins in cells equipped with the corresponding receptors (6). Both constitutive repression and hormone induction can be reproduced with a relatively short region of the MMTV long terminal repeat (LTR) region extending from the start of transcription up to about position −200. This region encompasses several binding sites for the hormone receptors, a binding site for the transcription factor nuclear factor I (NFI), and two octamer motifs, as well as the TATA box and initiation regions (6). The binding sites for the hormone receptors are imperfect hormone-responsive elements (HREs) deviating considerably from the consensus (68) and cover the region between −190 and −80 (59). Mutation in any of the sites has a more dramatic effect on hormone induction than it has on binding of the receptors to the MMTV DNA, suggesting a synergistic interaction among DNA-bound receptor molecules in vivo (13). In particular, mutations of one of the two central HREs, HREs 2 and 3, almost completely abolish hormonal induction, demonstrating that the various HREs are only fully operational as components of an integrated hormone-responsive region (HRR) (13). Mutation of the NFI-binding site reduces hormone induction by an order of magnitude without affecting receptor binding to the HRR (9, 10, 38), and mutation of the octamer motives also reduces induction, although to a lesser extent (8, 66).

The interplay between hormone receptors, NFI, and OTF-1/Oct-1 on free DNA is complex. Whereas a synergism between receptors and OTF-1/Oct-1 has been described in terms of DNA binding and cell-free transcription (8), NFI and hormone receptors do not cooperate but rather compete for binding to their respective sites on free promoter DNA (9). Binding of NFI also prevents access of OTF-1/Oct-1 to the octamer distal motif in vitro, suggesting steric hindrance between these two transcription factors (40). The study of these complex interactions in animal cells is hampered by the constitutive expression of members of the CTF/NFI and OTF-1/Oct-1 families in most of the cell lines analyzed. The situation is particularly complex for NFI, since members of this family are en-

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coded by at least three different genes that give rise to many different polypeptides through alternative splicing, and the various polypeptides can form homo- and heterodimers (31, 57). For OTF-1/Oct-1, in addition to the synergism described above, a transcriptional interference with glucocorticoid receptor (GR) has been reported (70), and these discrepancies may reflect cell-type effects.

An additional aspect influencing the interaction among transcription factors on the MMTV promoter is its organization in chromatin. It is known that the MMTV LTR is organized in positioned nucleosomes and that the chromatin structure around the HREs is altered after hormone induction (54). Since the MMTV promoter sequences are able to position a nucleosome in vitro (46, 53), it has been postulated that nucleosome organization controls access of transcription factors to the promoter (53, 54). In reconstituted nucleosomes containing the MMTV promoter region, the DNA double helix is rotationally positioned, and the orientation of the major groove permits binding of the hormone receptors to the HREs 1 and 4, while precluding binding to the central HREs 2 and 3 (45, 52). Moreover, binding of NFI to its cognate site is also precluded in the nucleosomally organized MMTV promoter (53).

A similar organization has been found in cells carrying chromosomally integrated single copies of the MMTV promoter (67). In this system, hormone induction leads to a remodelling of the MMTV nucleosome covering the HRR, leading to exposure of sequences around the nucleosomal dyad axis and to a full loading with hormone receptors, NFI, and OTF-1/Oct-1 (67). The mechanism by which hormone receptor binding induces remodelling of MMTV chromatin is unknown. There are reports suggesting an interaction of histone H1 with the NFI recognition sites (55, 74), and a relative depletion of histone H1 has been found over the MMTV promoter chromatin after hormone induction (7). Therefore, one mechanism of chromatin remodelling could involve hormone-induced depletion of linker histones.

To study the role of chromatin structure in modulating the interplay among transcription factors on the MMTV promoter, we have introduced MMTV reporter genes in the yeast *S. cerevisiae* in conjunction with expression vectors for relevant transcription factors. Since yeast cells have no linker histones, in case the regulation of MMTV is defective, one could study the effect of introducing histone H1 from metazoa (34). To simplify the analysis, and because it has been reported that OTF-1/Oct-1 does not function in yeast cells (64), we have limited our studies to the role of hormone receptors and NFI. The transcriptional regulation we observed turns out to be similar to that found in animal cells, in that the promoter is repressed in the absence of hormone and becomes activated after hormone treatment in an NFI-dependent fashion. The transactivation function located in the N-terminal half of the receptor (activator function 1 [AF1]) appears to be essential for induction. Repression seems to be mediated by a nucleosome positioned over the HREs and the NFI-binding site. These results do not support an essential role of histone H1 in the transcriptional control of the MMTV promoter and underline the usefulness of *S. cerevisiae* as a genetically manipulable system for understanding the mechanism underlying hormonal regulation of gene expression.

MATERIALS AND METHODS

Plasmids. Plasmid pLGZ-MMTV (2μm *URA3*) consists of a *Sal*I-*Xho*I fragment from pMC3.1 (25) containing the −236 to +111 promoter sequence of MMTV LTR, cloned into pLGZ (71) immediately upstream of the *lacZ* gene (Fig. 1). Plasmid pSCh113 was constructed by cloning the *Sal*I-*Xho*I fragment from pNF(D$^-$) (8), containing the MMTV LTR sequence with a mutated NFI-binding site, into pLGZ. Plasmid pSCh114 (2 μm *URA3*) is derived from pLGZ by replacement of the most distal part of the *CYC1* promoter (upstream from the *Sph*I site in position −70) by a −236 to −30 PCR fragment of the MMTV LTR (Fig. 1). Plasmid pSCh132 (*CEN6 ARS4 LYS2*) carries a *Sal*I-*Sca*I fragment from pLGZ-MMTV, including the MMTV-*lacZ* fusion, cloned into pRS317 (63). The 3xGRE-*lacZ* reporter plasmid pSX26.1 (2 μm *URA3*) was described by Schena and Yamamoto (61).

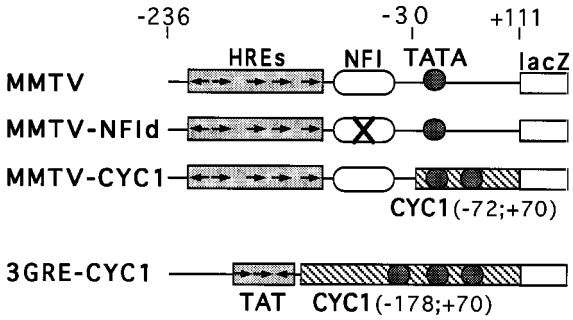

FIG. 1. Promoter-*lacZ* fusions used as reporters. A −236 to +111 fragment from the MMTV LTR fused to *lacZ* was used as the wild-type MMTV reporter. Both pLGZ-MMTV and pSCh132 plasmids, which differ in their yeast origin of replication (see Materials and Methods), contain this construct. An analogous construct carrying a mutation in the distal part of the NFI-binding site was denoted MMTV-NFId. MMTV-*CYC1* is a hybrid promoter consisting of the −236 to −30 fragment of MMTV, lacking the TATA box, fused to the proximal part of the *CYC1* yeast promoter, containing its two main TATA boxes. The 3xGRE-*CYC1* (3GRE-CYC1) reporter consists of a 26-bp fragment from the tyrosine aminotransferase gene, containing three GRE elements, fused to a −178 to +70 fragment from the yeast *CYC1* gene.

pAA-CTF2 (2 μm *LEU2*) (3) is an expression vector for pig NFI, derived from pAAH5 (4).

Plasmid pRS314N795 (*CEN6 ARS4 TRP1*) is an expression vector for rat GR (75) derived from pRS314 (*CEN6 ARS4 TRP1*) (62). Plasmids pGN795 (2μm *TRP1*), pGN556a (2μm *TRP1*), pGN525 (2μm *HIS3*), pG407C (2μm *TRP1*), and pGX556b (2μm *TRP1*), which express, respectively, wild-type and mutant (having deletions) forms of rat GR, have been described (61, 75) and are based on pG1 (2μm *TRP1*) (60). Plasmid pSCh129 (*CEN6 ARS4 LYS2*) carries a *Xho*I-*Sac*I fragment of pRS314N795, including the *GPD* promoter-rat GR gene fusion, cloned into pRS317.

Yeast strains and media. Strains are detailed in Table 1. To generate SChY10 to SChY31, W303-1A was transformed with the indicated plasmids. Strains SChY32 to SChY37 were also obtained by transformation of YPH499 with the specified plasmids. To obtain SChY39, LNZ12 was transformed to Leu$^-$ Ura$^+$ with *Bgl*II-digested plasmid pNKY85 (*leu2*::*hisG URA3 hisG*) (1). Leu$^-$ Ura$^-$ colonies were then isolated on selective medium containing 5-fluoroorotic acid (5-FOA). Strains SChY38, SChY40, SChY41, and SChY42 were generated by transformation of PSY142, SChY39, SLL5, and SLL9, respectively, with the indicated plasmids.

Yeast strains were grown in YEPD (1% yeast extract, 2% Bacto-Peptone, 2% glucose) or SD (0.67% yeast nitrogen base, 2% glucose) medium. Amino acids or purine-pyrimidine supplements were added at the levels described by Kaiser et al. (24).

Transcription analysis. Basal and hormonally induced transcription was determined by β-galactosidase assay and RNase mapping (77). For the β-galactosidase assay, yeast cells were grown overnight at 30°C in the appropriate selective media, subcultured by inoculation of fresh medium at an optical density of 0.1 at 600 nm, treated with ligand or with vehicle, and grown for an additional 8 h. Cells were then harvested and assayed for β-galactosidase after permeabilization (20). For RNase analysis, yeast cultures grown until mid-log phase in selective medium were treated for 4 h with ethanol or with ligand at the concentration indicated, and total RNA was prepared (28). Fifty micrograms of total RNA was hybridized to 3×10^5 cpm of a riboprobe that contained the −73 to +111 MMTV sequence. The reaction products of RNase mapping were then resolved on 6.5% sequencing gels.

Mapping of DNase I-hypersensitive sites. Approximately 10^9 yeast cells from a culture grown to mid-log phase in selective medium were incubated with ethanol or with 1 μM deacylcortivazol (DAC) for 120 min. Cells were then harvested and protoplasts were prepared by being treated with Zymolyase 100T (Seikagaku Co., Tokyo, Japan) in 1 M sorbitol–0.035% β-mercaptoethanol for 1 h at 30°C with mild agitation and lysed according to a modification of the protocol described by Fedor and Kornberg (17). Protoplasts were washed twice with cold 1 M sorbitol–1 mM EDTA–150 mM NaCl–1 mM phenylmethylsulfonyl fluoride–20 mM Tris-HCl (pH 8.0) and resuspended in 200 μl of cold 1 mM EDTA–150 mM NaCl–1 mM phenylmethylsulfonyl fluoride–0.2% Triton

TABLE 1. Yeast strains used in this study

Strain	Genotype	Source or reference
W303-1A	**a** *ade2-1 can 1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	56
YPH499	**a** *ade2-101 his3-Δ200 leu2-Δ1 trp1-Δ1 ura3-52 lys2-801*	62
PSY142	**a** *leu2 ura3 lys2-801*	A. Rich
LNZ12	As PSY142 but *zuo1*::*LEU2*	A. Rich
SLL5	**a** *leu2 trp1-1 ura3-52 his4-912*δ	33
SLL9	As SLL5 but *spt2*Δ	33
SChY10	As W303-1A but with pLGZ-MMTV, pAAH5, and pRS314	This study
SChY11	As W303-1A but with pLGZ-MMTV, pAA-CTF2, and pRS314	This study
SChY12	As W303-1A but with pLGZ-MMTV, pAAH5, and pRS314N795	This study
SChY13	As W303-1A but with pLGZ-MMTV, pAA-CTF2, and pRS314N795	This study
SChY14	As W303-1A but with pSX26.1 and pRS314	This study
SChY15	As W303-1A but with pSX26.1 and pRS314N795	This study
SChY16	As W303-1A but with pSCh113, pAAH5, and pRS314N795	This study
SChY17	As W303-1A but with pSCh113, pAA-CTF2, and pRS314N795	This study
SChY18	As W303-1A but with pLGZ-MMTV, pAAH5, and pGN795	This study
SChY19	As W303-1A but with pLGZ-MMTV, pAA-CTF2, and pGN795	This study
SChY20	As W303-1A but with pLGZ-MMTV, pAAH5, and pGN556a	This study
SChY21	As W303-1A but with pLGZ-MMTV, pAA-CTF2, and pGN556a	This study
SChY22	As W303-1A but with pLGZ-MMTV, pAAH5, and pGN525	This study
SChY23	As W303-1A but with pLGZ-MMTV, pAA-CTF2, and pGN525	This study
SChY24	As W303-1A but with pLGZ-MMTV, pAAH5, and pGX556b	This study
SChY25	As W303-1A but with pLGZ-MMTV, pAA-CTF2, and pGX556b	This study
SChY26	As W303-1A but with pLGZ-MMTV, pAAH5, and pG407C	This study
SChY27	As W303-1A but with pLGZ-MMTV, pAA-CTF2, and pG407C	This study
SChY28	As W303-1A but with pSCh114, pAAH5, and pRS314	This study
SChY29	As W303-1A but with pSCh114, pAA-CTF2, and pRS314	This study
SChY30	As W303-1A but with pSCh114, pAAH5, and pRS314N795	This study
SChY31	As W303-1A but with pSCh114, pAA-CTF2, and pRS314N795	This study
SChY32	As YPH499 but with pLGZ-MMTV, pAA-CTF2, and pRS314N795	This study
SChY33	As YPH499 but with pSCh132, pAA-CTF2, and pRS314N795	This study
SChY34	As YPH499 but with pLGZ-MMTV, pAAH5, and pRS314	This study
SChY35	As YPH499 but with pSCh132, pAAH5, and pRS314	This study
SChY36	As YPH499 but with pSCh132, pAA-CTF2, and pGN795	This study
SChY37	As YPH499 but with pSCh132, pAA-CTF2, and pG1	This study
SChY38	As PSY142 but with pLGZ-MMTV, pAA-CTF2, and pSCh129	This study
SChY39	As LNZ12 but *leu2*::*hisg*	This study
SChY40	As SChY39 but with pLGZ-MMTV, pAA-CTF2, and pSCh129	This study
SChY41	As SLL5 but with pLGZ-MMTV, pAA-CTF2, and pRS314N795	This study
SChY42	As SLL9 but with pLGZ-MMTV, pAA-CTF2, and pRS314N795	This study

X-100–20 mM Tris-HCl (pH 8.0). DAC or vehicle (1 μM each) was added to all of the buffers used during protoplast preparation and washes. Lysates were immediately digested with 0 to 200 U of DNase I (Boehringer, Inc.) in 2 ml of DNase I digestion buffer (65) for 120 s at 37°C. Reactions were stopped by addition of EDTA, sodium dodecyl sulfate, Tris-HCl (pH 8.8), and proteinase K to final concentrations of 12 mM, 0.5%, 50 mM, and 0.5 mg/ml, respectively. Proteolysis was allowed to proceed for 30 min at 37°C, and DNA was subsequently purified as described previously (50). For naked DNA controls, genomic DNA prepared from the corresponding strain was treated with 0.25 to 8 mU of DNase I under the same conditions. DNase I-cleaved genomic DNA samples were restricted with *Cla*I, resolved in 1.5% agarose gels, blotted onto a Quiabrane nylon-plus membrane, and probed with a 200-bp PCR fragment corresponding to the sequence of the *Escherichia coli lacZ* gene situated immediately upstream of the *Cla*I site. Genomic DNA cleaved by *Cla*I and *Sac*I (position −108) or *Dra*I (position −198) served as an internal size marker.

Resistance to micrococcal nuclease. Micrococcal nuclease-cleaved DNA samples were obtained as described above for DNase I hypersensitivity, except that lysate protoplasts were treated in the appropriate buffer (2) with 40 to 640 mU of micrococcal nuclease (Sigma, St. Louis, Mo.) for 20 min at 37°C. The purified DNA samples were resolved in a 1.2% agarose gel, blotted onto a Quiabrane nylon-plus membrane, and probed with a 96-bp PCR fragment covering MMTV sequences from −173 to −78.

Genomic footprinting. (i) DNase I treatment. DNase I treatment was performed as described for the mapping of DNase I-hypersensitive sites (described above) with 100 to 400 U of DNase I for 120 s at 37°C. Reactions were stopped as described above, and DNA was purified as described above. For naked DNA controls, genomic DNA prepared from the corresponding strain was treated with 0.25 to 8 mU of DNase I under the same conditions.

(ii) Micrococcal nuclease treatment. Micrococcal nuclease treatment was performed as described above but with 25 to 100 mU of enzyme for 120 s at 37°C. Reactions were stopped and DNA was purified as described above. For naked DNA controls, genomic DNA prepared from the corresponding strains was treated with 0.4 to 3.2 mU of micrococcal nuclease under the same conditions.

(iii) Linear PCR. DNase I- or micrococcal nuclease-cleaved DNA samples were further purified through a Nucleobond AX100 column. One microgram (single-copy MMTV strains) or 5 μg (multicopy MMTV strains) of clean DNA was then used as a template for a 50-cycle linear PCR with a radioactively labeled oligonucleotide, A-25 (AGGATAAGTGACGAGCGGAGACGGG). Stoffel fragment was used instead of conventional *Taq* polymerase. Amplified DNA corresponding to chromatin and naked DNA samples, equally digested as previously judged on an agarose gel, were run on 6.5% sequencing gels.

(iv) DMS treatment and ligation-mediated PCR. Approximately 10^9 yeast cells from a culture grown to mid-log phase in selective medium were incubated with ethanol or with 1 μM DAC for 120 min. Cells were then rapidly harvested and resuspended in freshly prepared 0.2% dimethyl sulfate (DMS)-containing medium. After 2 min of incubation at room temperature, reactions were stopped by washing of the cells four times with water, and DNA was isolated as described above. Piperidine cleavage of methylated DNA was performed as described previously (48). The resulting DNA (equally digested as judged from agarose gel electrophoresis) was used as a template for ligation-mediated PCR according to previously described conditions (67).

Gel scanning. Dried gels were analyzed with a PhosphorImager (Molecular Dynamics, Inc.) and ImageQuant software v3.0. Films were scanned with an Epson GT-8000 device.

RESULTS

Transcription from the MMTV promoter in yeast cells requires both activated hormone receptor and NFI. We have

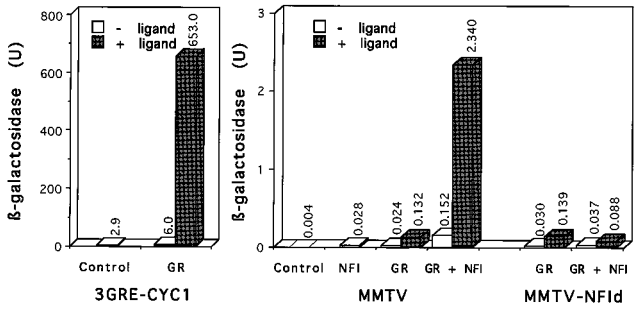

FIG. 2. MMTV induction by GR, ligand, and NFI. β-Galactosidase activities of mid-log-growth-phase cultures of strains (from left to right) SChY14, SChY15, SChY10, SChY11, SChY12, SChY13, SChY16, and SChY17 after 8 h of incubation with 0.1 μM DAC or vehicle. Reporter constructs and transactivators present in each strain are indicated. Mean values of a minimum of three experiments are given. Variation did not exceed 30%. 3GRE-CYC1, 3xGRE-*CYC1* reporter.

previously shown that the DNA sequences of the MMTV promoter between −230 and +115 adopt a precise position on the surface of a histone octamer (53, 67). Therefore, we have used these sequences for the initial experiments, rather than a combination of the HRR with a yeast minimal promoter. When a reporter containing the MMTV sequences linked to the *lacZ* gene of *E. coli* (Fig. 1, MMTV-*lacZ*) was introduced in *S. cerevisiae*, it was virtually silent (Fig. 2). In fact a 3xGRE-*CYC1-lacZ* reporter (Fig. 1), used by Schena and Yamamoto for the first experiments with GR in yeast cells (61), showed a much higher level of β-galactosidase activity (2.9 U) than MMTV-*lacZ* (<0.01 U).

The activity of the MMTV-*lacZ* reporter was only slightly enhanced by expression of rat GR in the absence of an agonistic ligand. Addition of the synthetic glucocorticoid DAC led to a weak increase in β-galactosidase activity (sixfold [Fig. 2]), suggesting that the MMTV promoter is not able to respond efficiently to an activated GR. That GR expressed in yeast cells is functional was demonstrated by the 110-fold effect of DAC on transcription from the 3xGRE-*CYC1* promoter driving the *lacZ* gene (Fig. 1 and 2). We conclude that GR cannot efficiently transactivate the MMTV promoter, probably because additional factors are required.

Transcription of the MMTV-*lacZ* reporter was only weakly enhanced by expression in yeast cells of the pig transcription factor NFI, which is known to mediate basal and induced transcription of MMTV in animal cells (9, 38, 41). Similar results were obtained with human CTF-1, a spliced variant of NFI containing a different carboxy-terminal end (3).

Expression of both GR and NFI in the absence of hormone did not cause a high level of expression of the MMTV-*lacZ* reporter (Fig. 2). However, treatment with the synthetic glucocorticoid DAC resulted in a significance enhancement (ninefold) of β-galactosidase activity (Fig. 2). Taken together, the simultaneous presence of NFI and the hormone-activated receptor produced a 600-fold enhancement of MMTV promoter activity (Fig. 2). The synergistic effect was mediated by the NFI-binding site, because mutation of this site leads to activation levels similar to those found in the absence of NFI (Fig. 1 and 2).

Since there are reports suggesting differences in the action spectra for various GR ligands in yeast and animal cells (18), we investigated the effect of various steroid analogs on MMTV induction in yeast cells (Fig. 3A). It is evident that triamcinolone acetonide, a classical synthetic glucocorticoid in animal cells, is less effective than RU28362 and DAC, which is the optimal inducer in yeast cells (18). For this reason, most experiments with GR were carried out with 0.1 μM DAC. Under these conditions, the dependence on NFI expression was more significant in strains expressing GR from a centromeric vector and at relatively low ligand concentrations than in cells expressing GR from episomal 2μm vectors at high ligand concentrations (not shown). The results suggest that at very high concentrations of active GR, a mechanism of transactivation independent of NFI can become operative.

Analysis of the RNA transcripts revealed the presence of one main initiation site at position +44, relative to the start site in animal cells (11), and several weaker initiation sites within a region of 40 to 50 bp (Fig. 3B). The distance from the TATA box of the main initiation site is characteristic of yeast promoters transcribed by RNA polymerase II (21, 22), suggesting that

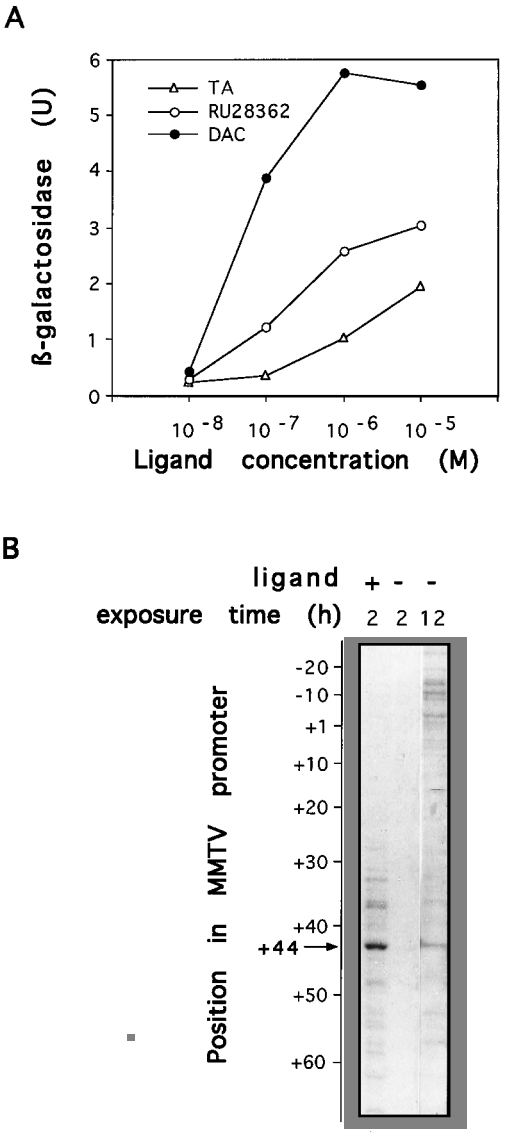

FIG. 3. Induction of MMTV promoter by different ligands and transcription start point. (A) β-Galactosidase activities of mid-log-growth-phase cultures of strain SChY13, expressing NFI and GR, after 8 h of incubation with triamcinolone acetonide (TA), RU28362, or DAC at the indicated concentration. (B) RNase mapping of MMTV transcription in strain SCh13 (MMTV, NFI, and GR). Total RNA was isolated from mid-log-growth-phase cultures treated for 4 h with 50 μM TA or vehicle. RNase mapping was performed as described in Materials and Methods. A 2-h exposure of a typical experiment is shown. An additional 12-h exposure of the uninduced RNA lane is also presented.

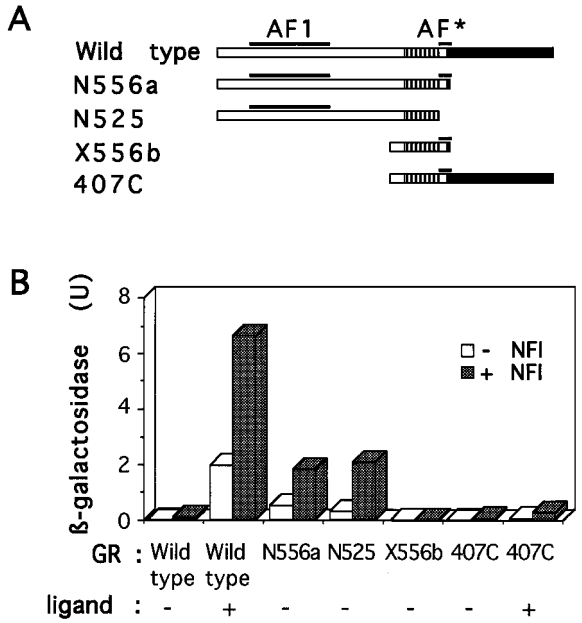

FIG. 4. Analysis of functional domains of GR. (A) Schematic representation of the wild-type and mutant (having deletions) variants of GR. (B) β-Galactosidase activities of mid-log-growth-phase cultures of strains (from left to right) SChY18, SChY19, SChY20, SChY21, SChY22, SChY23, SChY24, SChY25, SChY26, and SChY27 after 8 h of incubation with 0.1 μM DAC or vehicle. Transactivators present in each strain are indicated. All strains contained the MMTV reporter. Mean values of a minimum of three experiments are given. Variation did not exceed 25%.

the MMTV TATA box is functional in yeast cells. Upon hormone induction, the total amount of transcripts increased 60-fold, but the majority of the transcripts were still initiated at +44 (Fig. 3B).

From these results, we conclude that the MMTV promoter is efficiently transcribed in yeast cells and is regulated in a manner similar to its regulation in animal cells. Its transcription is dependent on binding of the activated hormone receptors to the HRR and is largely mediated by an interaction of NFI with its cognate sequence on the promoter.

The N-terminal activation domain of GR is required for optimal induction. To define the domains of GR involved in transactivation of the MMTV promoter in yeast cells, we used a series of constructions expressing various truncated forms of GR (Fig. 4A). A constitutive variant of rat GR, N556a, lacking the steroid binding domain (19), was also able to transactivate the MMTV-*lacZ* reporter in an NFI-dependent manner, though to a lesser extent than the complete GR. However, this construct includes a region, AF*, also called τ_2, which has been identified as an independent activation function (19, 23). Deletion of this short region in N525 does not reduce the level of induction, suggesting that the transactivation function at the N-terminal half (AF1), in combination with the DNA binding domain, can mimic the effect of the complete rat GR in terms of NFI dependence (Fig. 4). Deletion of the N-terminal half almost completely abolished transactivation, although a very weak effect of NFI was still detectable. A truncated GR containing the DNA binding domain and short flanking regions, X556b, was completely inactive. Therefore, AF* seems to be unable to efficiently activate transcription from the MMTV promoter on its own, although it may cooperate with AF1 in the intact GR.

The MMTV regulatory region confers hormone- and NFI-dependent transcription to a heterologous TATA box. Since the TATA box of the MMTV promoter differs from characteristic yeast core promoters, we linked the region between −236 and −30 of the MMTV promoter to the proximal part of the TATA box region of the yeast *CYC1* promoter driving the *lacZ* gene (Fig. 1). The levels of β-galactosidase activity observed with this construct were higher than those with MMTV-*lacZ*, but the activity was still dependent on NFI, GR, and ligand (Fig. 5). The main difference between MMTV and MMTV-*CYC1* was its dependence on NFI for activation. When MMTV-*CYC1* was used as a reporter, significant levels of β-galactosidase activity were detected in the absence of NFI expression, suggesting that the receptors may interact more efficiently with components of the basal transcriptional machinery in the context of the *CYC1* promoter.

A positioned nucleosome covers the HRR and the NFI-binding site. The chromatin structure of the MMTV promoter sequences in two strains carrying different constructs was investigated to test the influence of copy number and context. Both the multicopy construct pLGZ-MMTV and the single-copy construct pSCh132 used for these studies showed a similar functional behavior in terms of response to NFI (not shown) and ligand (Fig. 6A). Despite the higher copy number of pLGZ-MMTV (10 to 15 copies per cell) than of pSCh132 (1 to 2 copies), similar β-galactosidase levels were observed, indicating that the single-copy vector is transcribed more efficiently.

High-resolution analysis of the cleavage pattern obtained in both single-copy and multicopy vectors at low concentrations of micrococcal nuclease showed a protected region extending from position −45 to position −190 and flanked by hypersensitive cleavage sites (Fig. 6C). This pattern could be assigned to a positioned nucleosome, B, which would encompass all of the HREs, the NFI site, and the distal octamer motif, as previously described for animal cells (67) and nucleosome reconstitution assays (52). The proximal border of nucleosome B is less protected against nuclease digestion and exhibits a series of cleavage sites abutting on a highly sensitive cluster of sites characteristic of linker DNA. While there is some variation in this region between single-copy and multicopy constructs, it is ev-

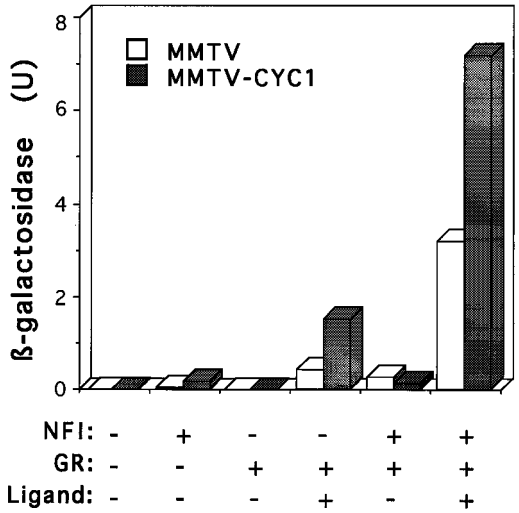

FIG. 5. Comparison of inducibility of MMTV and MMTV-*CYC1* constructs. β-Galactosidase activities of mid-log-growth-phase cultures of strains (from left to right) SChY10, SChY28, SChY11, SChY29, SChY12, SChY30, SChY13, and SChY31 after 8 h of incubation with 0.1 μM DAC or vehicle. Reporter constructs and transactivators present in each strain are indicated. Mean values of a minimum of three experiments are given. Variation did not exceed 30%.

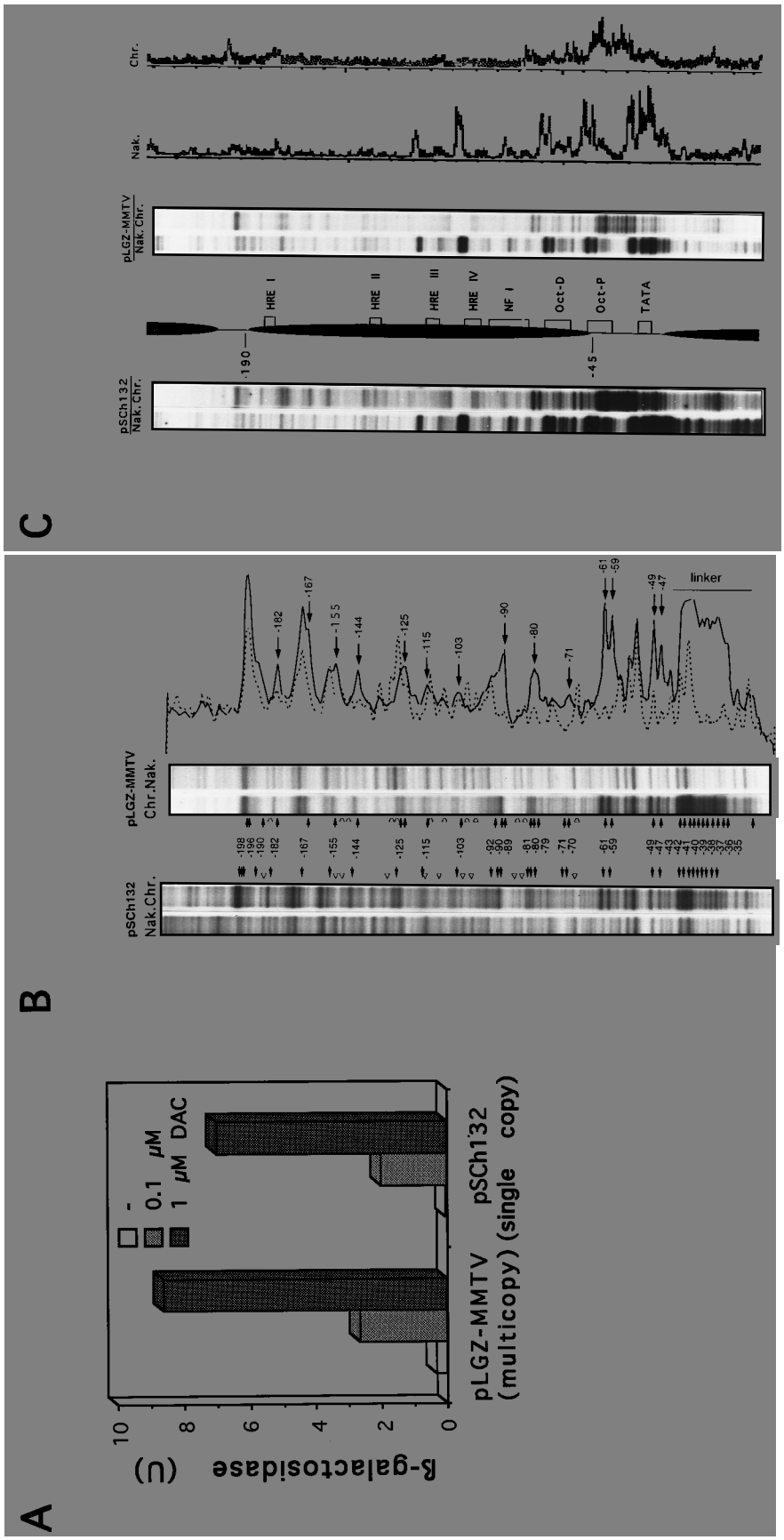

FIG. 6. Translational and rotational organization of MMTV chromatin. (A) Comparison of pLGZ-MMTV and pSCh132 inducibility. β-Galactosidase activities of mid-log-growth-phase cultures of strains SChY32 and SChY33 after 8 h of incubation with the indicated concentration of ligand. Both strains express NFI and GR. Mean values of a minimum of three experiments are given. Variation did not exceed 30%. (B) Rotational phasing of DNA on the MMTV promoter. In vivo DNase I footprinting of MMTV in strains SChY33 (left) and SChY32 (right) was performed as described in Materials and Methods. Nak., naked DNA treated with DNase I in vitro; Chr., DNA from chromatin of permeabilized spheroplasts treated with DNase I. Arrows and numbers indicate the hypersensitive sites. The open triangles indicate positions that are protected against DNase I in chromatin. Densitograms of the chromatin (continuous line) and naked DNA (stretched line) lanes of pLGZ-MMTV are shown on the right. (C) Translational positioning of nucleosomes on the MMTV promoter. In vivo micrococcal nuclease footprinting of MMTV in strains SChY33 (left) and SChY32 (right) was performed as described in Materials and Methods. Nak., naked DNA treated with micrococcal nuclease in vitro; Chr., DNA from chromatin of permeabilized spheroplasts treated with micrococcal nuclease. The regions of known transcription factor-binding sites and the borders of nucleosome B are indicated. Densitograms of the pLGZ-MMTV lanes are shown on the right.

ident that the linker encompasses the proximal octamer motif and the TATA box region. A protection further downstream, particularly evident in single-copy vectors, corresponds presumably to another positioned nucleosome, A, encompassing the initiation of transcription and the transcribed sequence of the LTR. The length of the linker between nucleosomes B and A is approximately 20 bp.

The upstream border of nucleosome B is also more sensitive to nuclease digestion, and there is a very strong cluster of cleavage sites at around −190 followed by another region of protection further upstream. This pattern probably reflects the existence of a shorter linker DNA region on the 5′ border of the LTR region, followed by another positioned nucleosome, which covers plasmid sequences. This array of positioned nucleosomes is clearly evident when the traces of the PhosphorImager quantitation of naked DNA and chromatin are compared (Fig. 6D). However, the scarcity of nuclease cleavage sites on this region of the LTR does not allow a precise determination of the length of this linker DNA.

The rotational orientation of the promoter sequences was nonrandom in both constructs, as demonstrated by the differences in DNase I cleavage patterns of chromatin compared with free genomic DNA (Fig. 6B). Apart from a cluster of very hypersensitive sites located over the linker DNA from −35 to −43, there is a reiteration of hypersensitive (arrows) and protected (triangles) sites recurring approximately every 10 bp. This pattern can be clearly seen in the superposition of the scans of the chromatin lane and the free DNA lane (Fig. 6B) and is the pattern expected for anisotropic DNA sequences, which adopt a dominant rotational setting on the surface of the histone octamer. The positions of the preferred cleavage sites correspond to those previously described in the chromatin of animal cells (67) and in reconstituted nucleosome B (52).

We conclude from these studies that the regulatory sequences of the MMTV promoter are organized into a nucleosome in yeast cells which adopts a translational and rotational positioning compatible with that previously found in animal cells. Therefore, the primary nucleotide sequence seems to be the main determinant for nucleosome phasing over the regulatory region of the MMTV promoter.

Changes of MMTV promoter upon hormone induction. Binding of factors to the MMTV promoter was studied only with the single-copy vector pSCh132 to ensure a more homogeneous hormone response. Given its higher level of transcriptional activity, this vector should generate a clear pattern of bound proteins (67). Treatment of intact cells with DMS followed by cleavage of the DNA at the methylated guanines with piperidine yields a pattern indistinguishable from that obtained with naked DNA (data not shown), suggesting that there are no proteins bound to the major groove of DNA in the absence of GR or NFI. This pattern is not altered significantly when GR and NFI are expressed in the absence of ligand (Fig. 7A). However, 2 h after addition of the hormone ligand, there is a clear protection of the two guanines within the NFI-binding site (Fig. 7A, triangle). In some experiments, weak protection and hypersensitivity of the guanines over the HREs were also detected. In the particular experiments shown in Fig. 7A, protection over the NFI site was 40% as determined by quantitation with a PhosphorImager, and there is clear hypersensitivity over a cryptic GR-binding site (arrow) (58), which we have recently identified as a functional HRE (68a). These results suggest an interaction of GR and NFI with a subpopulation of MMTV promoters.

Micrococcal nuclease digestion was used to investigate changes in the general nucleosomal organization of the MMTV promoter after hormone induction. A comparison of the digestion products separated on nondenaturing agarose gels and visualized with a probe corresponding to nucleosome B sequences (−173 to −78) does not detect significant changes of the nucleosome ladder (Fig. 7B). The typical yeast nucleosomal spacing of 160 to 170 bp was observed in the uninduced state and in the induced state, suggesting that there are no major chromatin rearrangements upon induction and that nucleosome B remains in place.

In animal cells, hormone induction leads to hypersensitivity of the dyad axis of nucleosome B against digestion by DNase I (67). We have investigated whether a similar change is observed when the MMTV promoter is induced in yeast cells. The results of an indirect end labelling experiment show that hormone induction causes hypersensitivity of the linker region flanking nucleosome B but causes no change in nuclease cleavage over the dyad axis (Fig. 7C). In addition, there is a region of DNase I hypersensitivity over nucleosome A. We conclude that hormone induction leads to changes in chromatin organization but cannot detect either exposure of the DNA sequences around the nucleosome B dyad, as previously reported for animal cells (67), or disruption of this nucleosome.

MMTV transcription in yeast strains lacking putative linker proteins. The results presented so far suggest that transcriptional control of the MMTV promoter in *S. cerevisiae* is similar to that in animal cells, despite the lack of histone H1 in yeast cells (34). There have been claims that other yeast proteins can fulfill the function of histone H1, in particular the HMG-1-like protein SPT2/SIN1 (30, 47) and the Z-DNA binding protein ZUO1 (76). We have investigated the behavior of the MMTV promoter in yeast strains deficient in either of the genes coding for these proteins. The results show that mutations in these genes do not increase basal activity of the promoter (Fig. 8), demonstrating that the corresponding proteins are not essential for maintaining the repressed state of the MMTV promoter in the absence of hormone. The hormone-dependent activation is also preserved in either *spt2/sin1* or in *zuo2* strains (Fig. 8). The only change observed is a weaker activation at a low hormone concentration in the *spt2/sin1* strain, which suggests a positive effect of SPT2/SIN1 in receptor action.

DISCUSSION

Function of MMTV *cis* elements in yeast cells. To study the behavior of the regulatory region of the MMTV promoter in yeast cells, we decided to include in our reporter constructs not only the HREs and the NFI-binding sites but also the TATA box and the initiator region. This decision was based on previous reports suggesting a role of these core promoter elements in regulating transcription (51, 66) and in our intention to reconstitute a yeast system as homologous to the murine situation as possible. Our results show that, in the presence of GR and NFI, the core MMTV promoter is efficiently transcribed in yeast cells after hormone induction. Several transcription start sites were detected, the main site of which is located at a distance from the TATA box characteristic of *S. cerevisiae*, which suggests that the MMTV TATA box is properly recognized by the basal transcriptional machinery of yeast cells. In the absence of ligand, transcription was very weak, but the same set of initiation sites was used. When the MMTV TATA box and initiation region were replaced by the corresponding regions of the *CYC1* promoter, the level of basal activity in the absence of ligand was higher, suggesting that either the MMTV TATA box is suboptimal in yeast cells or that it is not accessible for the relevant factors. The latter explanation is not unlikely, since deletion of the HRR leaving the NFI site and all the sequences downstream intact led to an

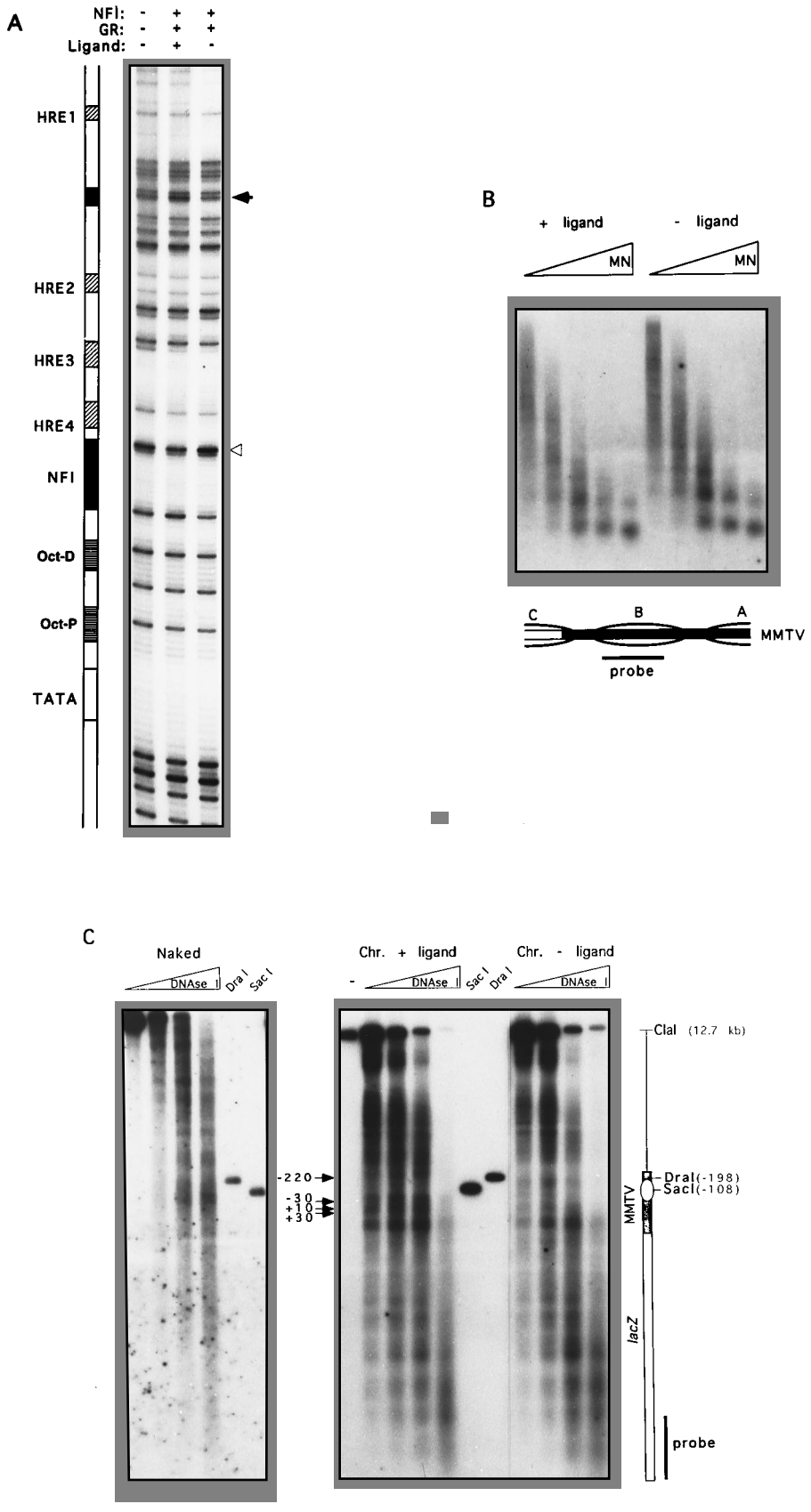
A
NFI: - + +
GR: - + +
Ligand: - + -
HRE1
HRE2
HRE3
HRE4
NFI
Oct-D
Oct-P
TATA
B
+ ligand
- ligand
MN
MN
C
B
A
MMTV
probe
C
Naked
DNAse I
DraI
SacI
Chr. + ligand
Chr. - ligand
-220
-30
+10
+30
ClaI (12.7 kb)
DraI(-198)
SacI(-108)
MMTV
lacZ
probe

FIG. 7. Changes on MMTV promoter upon hormone induction. (A) DMS genomic footprinting. SChY36 (MMTV, NFI, and GR) and SChY37 (MMTV) cells were grown in selective medium to mid-log growth phase, incubated for 2 h with 1 μM DAC or vehicle, and treated with 0.2% DMS for 2 min. Methylated DNA was processed as described in Materials and Methods. The open triangle indicates the position of guanines that are protected, and the solid arrowhead indicates the position of guanines that are hypermethylated in hormone-treated cells. (B) Influence of hormone induction on the sensitivity of the MMTV promoter to micrococcal nuclease. Mid-log-growth-phase cultures of SChY36 (MMTV, NFI, and GR) were treated for 2 h with 1 μM DAC or vehicle. Permeabilized spheroplasts obtained from these cells were incubated with different amounts of micrococcal nuclease (MN) and processed as described in Materials and Methods. The resulting genomic DNA was resolved in an agarose gel, transferred to a nylon membrane, and hybridized with a PCR-labeled probe covering MMTV sequences from −173 to −78, as shown in the scheme at the bottom. (C) Induction of DNase I-hypersensitive sites on the MMTV promoter. SChY36 (MMTV, NFI, and GR) cells, grown to mid-log phase in selective medium, were incubated for 2 h with 1 μM DAC or vehicle. Permeabilized spheroplasts or naked DNA obtained from these cells was incubated with different amounts of DNase I and processed as described in Materials and Methods. The resulting genomic DNA was restricted with *Cla*I, resolved in an agarose gel, transferred to a nylon membrane, and hybridized with a PCR-labelled probe covering the *lacZ* sequence immediately upstream of the *Cla*I site, as shown in the scheme on the right. The position of nucleosome B on the MMTV promoter is indicated by an oval. Hypersensitive sites are indicated by arrows. Chr., chromatin.

increase in transcription in the absence of GR or ligand (10a). Moreover, a reporter containing the *CYC1* TATA region and synthetic GREs also had a much higher level of activity in the absence of ligand (61), supporting the notion that the MMTV HREs are particularly efficient in repressing transcription in the uninduced state. It has been suggested that yeast endogenous proteins could interact with HREs and mediate repression of such reporters (61), but we have not found any evidence for proteins bound to the HREs in the absence of activated receptors (described below). The MMTV-*CYC1* reporter responded to hormone induction but was less strictly dependent on NFI for transcription, as if the *CYC1* TATA region was less tightly controlled by the upstream HREs. However, since no structural data were obtained with the hybrid promoter, we cannot provide a conclusive mechanistic explanation for the differences observed.

Mutation of the NFI site led to a dramatic reduction in ligand-dependent transactivation of the MMTV promoter, particularly at low concentrations of inducer. This suggests that, under these conditions, hormone-dependent transactivation is mainly mediated through a functional synergism between GR and NFI. The nature of this synergism can only be indirect, because no indication for direct synergism between the two proteins has been detected in DNA binding experiments or in cell-free transcription assays (9, 40). Since at high concentrations of inducer the dependence on NFI was relaxed, we assume that activated GR can interact directly with the basal transcription machinery and that one function of NFI is to facilitate this interaction at low concentrations of activated GR.

Mutations in the octamer motifs had a complex phenotype, probably due to redundancy of the elements and to the generation of artificial TATA boxes (data not shown). However, a mutation in the octamer distal motif, which is known to eliminate the contribution of OTF1/Oct1 to MMTV transcription in animal cells (8), did not influence the activity of the MMTV promoter in yeast cells (13a). This finding suggests that the octamer motifs are not operative in yeast cells, probably because of the lack of endogenous octamer transcription factors. A detailed analysis of the role of recombinant OTF1/Oct1 in yeast cells is now in progress.

Role of transactivators. In our studies, we have left out the function of OTF1/Oct1, essentially because this factor has been claimed to be inactive in yeast cells (64). There could be several reasons for this, but one possibility is the lack of appropriate coactivators such as OBF-1, which mediates expression of the immunoglobulin promoter in lymphoid B cells (64). However, since there are indications for the existence of two independent pathways for MMTV activation by GR, one involving NFI and the other mediated by OTF1/Oct1 (40), we focused on the synergism between GR and NFI.

GR has been shown to mediate transactivation of yeast promoters containing GREs in response to appropriate ligands (61). We confirm these findings and extend them by showing that GR is also able to transactivate the intact MMTV promoter with its own TATA box. Since, as shown above, this activation is highly dependent on NFI, it was interesting to know which of the known transactivation functions of GR are relevant for this effect. Our studies show that the amino-terminal region of the receptor linked to the zinc fingers is sufficient to generate a significant NFI-dependent transactivation. Since the zinc fingers alone are inactive, it seems that the transactivation domain, AF1 or τ_1, is responsible for the effect we see with the complete GR. The transactivation functions located on the hinge region, τ_2, or on the very carboxy-terminal region, AF2, are not operative on their own, although they could possibly synergize with the N-terminal activity in the presence of ligand. Our results do not differ from those obtained with other GRE-dependent reporters in yeast cells (61, 73).

It has been previously found that overexpression of GR τ_1 in yeast cells leads to squelching and growth arrest (73) and that τ_1 can interact with components of the basal transcriptional machinery (36). It is likely that this interaction mediates the NFI-independent transactivation of the MMTV promoter at high concentrations of inducer.

Although induction of GRE-containing reporters is ligand dependent, the synthetic glucocorticoids used routinely in animal cells, such as dexamethasone and triamcinolone acetonide, are only relatively inefficient in yeast cells (18). Although transactivation of the MMTV promoter involves more complex interactions, we also observed a similar dependence on the nature of the added ligand, with the optimal inducer being DAC. Whether this behavior is due to the existence of an ATP-dependent ligand transporter (29) or to the overexpres-

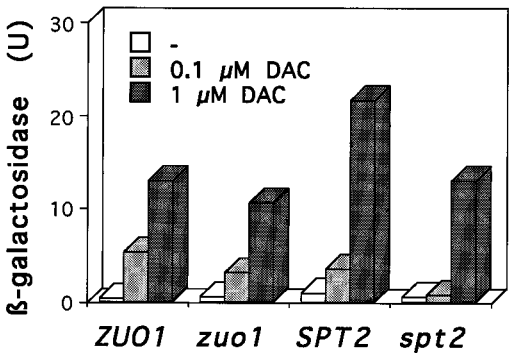

FIG. 8. Influence of putative linker proteins on MMTV transcription. β-Galactosidase activities of mid-log-growth-phase cultures of strains SChY38 (*ZUO1*), SChY40 (*zuo1*), SChY41 (*SPT2*), and SChY42 (*spt2*) after 8 h of incubation with the indicated concentration of ligand are shown. All strains express NFI and GR. Mean values of a minimum of three experiments are given. Variation did not exceed 20%.

sion of GR (72) remains to be proven. However, a single point mutation in the ligand binding domain is sufficient to generate a GR in yeast with ligand dependence similar to that found in animal cells (18).

The NFI-binding site of the MMTV promoter does not seem to be recognized by transcription factors from *S. cerevisiae*, since coexpression of recombinant NFI is essential for transactivation by the ligand-activated GR. Therefore, HAP1, HAP2, and HAP3 factors, which are known to recognize CAAT boxes (15, 42, 43, 49), do not appear to interact with the MMTV promoter and do not interfere with NFI action. In fact, the HAP factors have turned out to be more related to the NF-Y family of transcription factors (16).

The NFI family of transcription factors is composed of a heterogeneous mixture of isoforms generated by transcription and alternative splicing from at least four different genes (31, 57). In yeast cells, the function of the various transactivation motifs on the carboxy-terminal half of NFI is complex, and a proline-rich domain appears to be inactive (3). In animal cells, this domain appears to contact TFIIB and TAFII55 rather than TBP (14, 27). The main transactivation function shows homology to the CTD repeat of RNA polymerase II and is located between amino acids 463 and 508 (26, 69). Most of our experiments were performed with an isoform of NFI, called CTF2, lacking some of these repeats and exhibiting poor transactivation in yeast cells (3). Despite this weak autonomous transactivation, CTF2 was able to synergize efficiently with GR on the MMTV promoter. Control experiments with CTF1, which contains the complete domain, gave essentially identical results. We have not made any attempt to map precisely the region of NFI involved in the synergistic transactivation of the MMTV promoter.

The HRR is precisely positioned in nucleosomes. Our main interest when introducing the MMTV promoter in yeast cells was to study the contribution of its chromatin organization in regulated transcription. Independently of the genetic background (2μm plasmids or centromeric plasmids), the region of the HRR was precisely organized in chromatin. A nucleosome, B, covered the region extending from −45 to −190, which has also been found to be organized into a nucleosome in animal cells (67) and in chromatin reconstitution experiments in vitro (53). Nucleosome B covers all HREs, the NFI-binding site, and the octamer distal motif. The octamer proximal motif and the TATA box are found in the linker between this nucleosome and nucleosome A, which covers the transcribed part of the LTR. This linker region is about 20 bp long, whereas the linker between nucleosome B and the upstream nucleosome, which covers vector sequences, is difficult to determine because of the lack of nuclease cleavage sites. This was probably due to the bacterial vector sequences, since a construct containing the complete MMTV LTR exhibited a more regular array of nucleosomes flanking the HRR with a spacing of 190 bp (13a). Thus, when the complete LTR is included, it imposes its spacing despite other yeast constraints. A similar observation has been made for the region between −140 and +314 of the *SNR6* gene. In the chromosomal context, a large region of the gene is organized into a phased array of nucleosomes, whereas when embedded in plasmid sequences, only the relevant promoter sequences, but not the flanking DNA, are regularly organized in nucleosomes (35). Since we are working with a metazoan promoter, we decided to study its structure in a plasmid context, to prevent influences from flanking chromatin structures. However, in future work, it may be interesting to test whether MMTV promoter sequences can compete with other yeast sequences for nucleosome positioning.

The position of nucleosome B was independent of the genetic background and of the exact nature of the flanking sequences, since these sequences were different in the single-copy and the multicopy vectors. The same nucleosome positioning was seen in yeast strains which did not express GR or NFI. Moreover, in DMS protection experiments, we could not detect bound proteins which could have served as a barrier signal for the positioning of nucleosomes. These results suggest that the primary nucleotide sequence of the MMTV promoter, rather than bound proteins, is the primary determinant of nucleosome positioning and that in a plasmid context, it imposes a nucleosome structure which overrides other constraints and yields the same spacing and translational nucleosome positioning as in animal cells. To our knowledge, this is the first report of animal DNA sequences able to organize nucleosomes in yeast cells.

The MMTV promoter sequences adopt a dominant rotational orientation on the surface of nucleosome B in chromatin reconstitution experiments (53) and in intact cells (67). A similar rotational setting of the DNA double helix was also found in the various yeast vectors. In such positioned DNA, the major groove of the internal HREs, the NFI site, and the distal octamer motif are oriented towards the histone octamer and are therefore unaccessible for factor binding. Removal of the HREs results in a higher level of accessibility of the NFI-binding site and enhanced transcription in the absence of activated GR (10a). These findings are compatible with a role of the HRR in mediating constitutive repression of the MMTV promoter by positioning a nucleosome over the NFI site and preventing binding of NFI. Whether this effect is due to the inclusion of NFI in a nucleosome or to the precise rotational setting of the DNA remains to be established.

Induction leads to factor binding without major chromatin rearrangements. In animal cells, hormone induction of the MMTV leads to an alteration in chromatin structure manifested by a higher accessibility to digestion by DNase I (54) and restriction endonucleases (32). We have recently found that in gently manipulated cells, the nuclease hypersensitivity induced by hormone treatment is restricted to a short DNA region overlapping the pseudo-dyad axis of nucleosome B and that this nucleosome remains in place when all receptor binding sites, the NFI site, and the octamer motifs are occupied (67). In yeast cells, we cannot find evidence for a nucleosome B disruption, under conditions in which we detect 40% occupancy of the NFI-binding site, suggesting that nucleosome B remains in place. In that respect, the MMTV reporter in yeast cells behaves like that in animal cells, in which recruitment of NFI is found after activation of GR and both transcription factors bind to the surface of a rearranged nucleosome B (67). A similar binding of transcription factors on the surface of a positioned nucleosome has been reported for the rat albumin enhancer (37).

Indications for a change in nucleosome organization in yeast cells were obtained by high-resolution analysis of MNase digestion products, in the form of a higher level of accessibility of the sequences around the TATA box. Similarly, in DNase I digestion experiments, the linker DNA regions flanking nucleosome B were more efficiently cleaved after hormone induction. However, no DNase I hypersensitivity was detected over the pseudo-dyad axis. This difference from the results obtained in animal cells could reflect the more extensive manipulation required for chromatin analysis in yeast cells, since manipulation of nuclei from animal cells also results in a less-focused region of DNase I hypersensitivity (67). Alternatively, it could be due to the absence in yeast cells of linker histones and, in particular, histone H1, which also contacts the pseudo-dyad axis (described below) (12). To test this hypothesis, one

could introduce histone H1 in *S. cerevisiae* and study the effects on MMTV transcription and chromatin organization. However, this type of experiment is hampered by the observation that histone H1 is rather toxic in yeast cells and leads to a rapid arrest in cell growth (34, 39).

Histone H1 and HMG-1-like proteins are not essential for MMTV regulation. Because histone H1 has been claimed to participate in MMTV regulation in animal cells (5, 7), we wanted to know whether yeast proteins functionally homologous to histone H1 were required for constitutive repression or hormonal induction. One possible candidate is zuotin, a protein with affinity for Z-DNA, which has been postulated to assume some functions of histone H1 (76). However, a yeast strain lacking zuotin exhibits normal transcriptional behavior from the MMTV promoter. Another candidate is the HMG-1-like protein SIN1, also called SPT2 (30, 33), but a deletion of the gene coding for this protein did not change the general properties of the MMTV promoter, except for a weaker response at low concentrations of inducer. This latter observation is interesting in view of reports claiming that metazoan HMG-1 enhances the DNA binding and function of the progesterone receptor (44). Moreover, *sin1*/*spt2* was isolated as a suppressor of *swi*/*snf* mutations, and several other suppressor mutations of this genotype affect genes involved in chromatin structure. However, except for this small effect at low concentrations of inducer, our results suggest that regulation of MMTV transcription can take place in the virtual absence of linker histones. In particular, histone H1 does not seem to be required for establishing constitutive repression of the MMTV promoter in yeast cells. Of course, this does not exclude the possibility that in animal cells, histone H1 plays a role in modulating the transcriptional behavior of the MMTV promoter.

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