Chromatin Remodeling and Control of Cell Proliferation by Progestins via Cross Talk of Progesterone Receptor with the Estrogen Receptors and Kinase Signaling Pathways

GUILLERMO P. VICENT,[a] CECILIA BALLARÉ,[a] ROSER ZAURIN,[a] PATRICIA SARAGÜETA,[b] AND MIGUEL BEATO[a]

[a]*Centre de Regulació Genòmica (CRG), Universitat Pompeu Fabra (UPF), PRBB, Dr Aiguader 88, 08003 Barcelona, Spain*

[b]*Instituto de Biología y Medicina Experimental, CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, 1428 Buenos Aires, Argentina*

ABSTRACT: Transcription from the mouse mammary tumor virus (MMTV) promoter can be induced by glucocorticoids or progestins. Progesterone treatment of cultured cells carrying an integrated single copy of an MMTV transgene leads to recruitment of progesterone receptor (PR), SWI/SNF, and SNF2h-related complexes to MMTV promoter. Recruitment is accompanied by selective displacement of histones H2A and H2B from the nucleosome B. In nucleosomes assembled on promoter sequences, SWI/SNF displaces histones H2A and H2B from MMTV nucleosome B, but not from other MMTV nucleosomes or from an rDNA promoter nucleosome. Thus, the outcome of nucleosome remodeling by purified SWI/SNF depends on the DNA sequence. On the other hand, 5 min after hormone treatment, the cytoplasmic signaling cascade Src/Ras/Erk is activated via an interaction of PR with the estrogen receptor, which activates Src. As a consequence of Erk activation PR is phosphorylated, Msk1 is activated, and a ternary complex PR-Erk-Msk1 is recruited to MMTV nucleosome B. Msk1 phosphorylates H3 at serine 10, which is followed by acetylation at lysine 14, displacement of HP1γ, and recruitment of Brg1, PCAF, and RNA polymerase II. Blocking Erk activation or Msk1 activity prevents induction of the MMTV transgene. Thus, the rapid nongenomic effects of progestins are essential for their transcriptional effects on certain progestin target genes. In rat endometrial stromal cells, picomolar concentrations of progestins trigger the cross talk of PR with ERβ that activates the Erk and Akt kinase pathways leading to cell proliferation in the absence of direct transcriptional

Address for correspondence: Dr. Miguel Beato, Center for Genomic Regulation (C.R.G.), PRBB, Dr Aiguader 88, E-08003 Barcelona, Spain. Voice: +34-93-224-0901; fax: +34-93-224-08-99.
e-mail: miguel.beato@crg.es

Ann. N.Y. Acad. Sci. 1089: 59–72 (2006).
doi: 10.1196/annals.1386.025

effects of the ligand-activated PR. Thus, depending on the cellular context rapid kinase activation and transcriptional effect play different roles in the physiological response to progestins.

KEYWORDS: mouse mammary tumor virus; progesterone receptor; chromatin; transcriptional regulation; histone H1; kinases

The basic unit of chromatin is the nucleosome, which contains a core of histones around which DNA is wrapped in 1.65 left-handed superhelical turns. This core contains two molecules of each of four core histone proteins: H2A, H2B, H3, and H4. Eukaryotic cells contain a fifth class of histones, called linker histones, which bind to the nucleosome and to the linker DNA and alter the stability with which DNA within the nucleosome is associated. The prototype of the linker histones is histone H1, of which there are six somatic isoforms and a testis-specific form. Modulation of the structure and dynamics of the nucleosome is an important regulatory mechanism of all DNA-based processes in eukaryotic cells, such as transcription, DNA replication and repair. Changes in chromatin structure affect the binding of nonhistone proteins, such as transcription factors and the replication machinery, by restricting access to the binding sites within the DNA.

The cell has developed multiple strategies for the optimal use of chromatin as the substrate for DNA-directed processes. The two more important remodeling mechanisms are ATP-dependent chromatin remodeling enzymatic complexes and enzymes that modify the histones posttranslationally. In addition, the cells can incorporate core histone variants to alter the structure and dynamics of specific chromatin regions. ATP-dependent chromatin remodeling complexes utilize the energy from ATP hydrolysis to rearrange both histone–DNA and histone–histone interactions. Most ATP-dependent chromatin remodeling factors are multisubunit complexes with an ATPase as the catalytic center. These ATPase subunits can be classified into three families on the basis of the presence of functional domains: the SWI2/SNF2-, the Mi-2/CHD-, and the ISWI-ATPases.[1] The SWI/SNF family comprises yeast Snf2 and Sth2, *Drosophila melanogaster* brahma (BRM), and mammalian BRM and brahma-like 1 (BRG1). These proteins are characterized by the presence of a bromo domain, which binds acetylated histones.[2] The imitation SWI (ISWI) family of enzymes have a SANT domain, which is thought to act as a histone-binding domain,[3] and may recognize specifically modified histones. There are two ISWI homologues in yeast (Isw1 and Isw2) and mammals (SNF2H and SNF2L). A third class, the chromodomain and helicase-like domain (CHD) family, is characterized by the presence of two amino-terminal chromodomains, which interact with methylated histone tails.[4]

Posttranslational modifications, primarily of the histone-tail domains, such as acetylation of lysines, methylation of lysines or arginines, and phosphorylation of serines and threonines, alter the properties of chromatin, influencing

accessibility of the DNA, and in addition, act as signals for the recruitment of nuclear factors.[5,6] Functional cooperation between histone-modifying and ATP-dependent chromatin-remodeling enzymes can mediate positive and negative output on gene regulation. For example, the bromodomain of Brg1 binds the H4 tail when acetylated at K8,[7] and TAFII250 binds the H3 tail when acetylated at both K9 and K14.[8]

Nuclear receptors (NRs) are one of the most abundant classes of transcriptional regulators in animals (metazoans). They regulate diverse functions, such as homeostasis, reproduction, development, and metabolism. NRs share structurally conserved domains and can be regulated through steroids, thyroid hormone, retinoic acid, vitamins, or other proteins. They function as transcription factors, often in complex with other coregulators, and govern transcription of target genes involved in such varied processes as homeostasis, reproduction, development, and metabolism.[9] The steroid/thyroid hormone receptors are members of a very large family of nuclear ligand-activated transcription factors that includes the steroid receptors (those for progesterone [PRs], androgen [ARs], estrogen [ERs], glucocorticoids [GRs], and mineralocorticoids) and receptors for thyroid hormone, retinoids, and vitamin D, as well as an even larger group of proteins termed orphan receptors, whose ligands and/or functions are as yet unknown.[10,11] These receptors play key roles both as transcriptional activators and as repressors in all aspects of biological function, including regulation of development, metabolism, and reproduction.

Control of transcription by steroid hormones often involves binding of the ligand-activated hormone receptors to promoter/enhancer regions of regulated genes followed by recruitment of coregulators, remodeling of chromatin, and formation of the transcription initiation complex. In some cases, regulation of transcription is based on an interaction of the hormone receptors with other sequence-specific transcription factors. But many regulatory regions of hormone-responsive genes contain binding sites for the hormone receptors (hormone-responsive elements [HREs]) and are organized in positioned nucleosomes, which are remodeled in the context of hormone induction. To elucidate these processes, we have studied the induction of the mouse mammary tumor virus (MMTV) promoter by the steroid hormone progesterone.

INTRODUCTION TO THE HORMONAL REGULATION OF THE MMTV PROMOTER

The organization of eukaryotic promoter and enhancer regions in chromatin also plays an essential role in modulating interactions of regulatory proteins and transcription factors with their DNA target sequences. The participation of chromatin dynamics in gene regulation has been studied in great detail. One of the more extensively characterized model systems is the hormonal regulation of the expression of the MMTV. During hormone induction of the MMTV

promoter, there are rapid changes in chromatin structure as evidenced by the appearance of a DNase I hypersensitive site in a region containing the HREs.[12] The MMTV HREs were the first to be identified in experiments with the glucocorticoid receptor (GR),[13–15] although they were later shown to bind the progesterone receptor (PR) with high affinity[16,17] and to mediate progesterone activation of transcription.[18]

The MMTV promoter is organized into positioned nucleosomes, with a nucleosome covering the HREs and the binding site for NF1[19] (FIG 1). A full hormonal activation of the promoter requires not only the HREs but also the NF1 binding site, indicating that both factors synergize *in vivo*.[16,20] However, in cell-free transcription experiments with MMTV promoter DNA, the hormone receptors activate transcription,[21] but no synergism with NF1 is detected. Instead NF1 competes with hormone receptors for binding and transactivation of naked DNA templates.[20] In intact cells, however, both hormone receptors and NF1 occupy their binding sites simultaneously after hormone induction on the surface of a nucleosome-like particle[22] (FIG 1). These results suggested an important role of the nucleosomal organization of the promoter for efficient induction.

In addition to PR and NF1, the octamer transcription factor 1 (Oct1) participates in MMTV regulation. In fact, a transcriptional synergism has been described between PR and Oct1 *in vitro* and in transient transfection experiments.[23] However, because these results have not been confirmed in studies with chromatin-organized MMTV promoter sequences, we will not further mention the role of Oct1 in this review.

When the MMTV promoter DNA is assembled into nucleosomes *in vitro*, it adopts a precise rotational orientation on the surface of the histone octamer that exposes the external HREs 1 and 4 but leaves inaccessible the central HREs 2, 3, and 5, which are essential for hormone induction.[24] Moreover, NF1 cannot bind to MMTV promoter sequences assembled into regular nucleosomes because it encircles the DNA double helix completely.[24,25] Therefore, we concluded that the nucleosome must experience changes during induction in order to enable the simultaneous binding of receptors and NF1 and to facilitate their functional synergism.

A few minutes after progesterone treatment of breast cancer cells carrying a single copy of the MMTV promoter integrated in their chromosomes, a characteristic and sharp DNase I hypersensitive site appears near the symmetry axis of the nucleosome encompassing the HREs.[22] The same hypersensitive site can be induced by treatment with moderate concentrations of inhibitors of histone deacetylases, such as sodium butyrate or trichostatin A,[26] suggesting that it reflected an "opening" of the chromatin that can be initiated by a moderate increase in histone acetylation. We have devoted our attention during the last years to understanding the nature of this hormone-induced change in chromatin structure and how it is brought about.

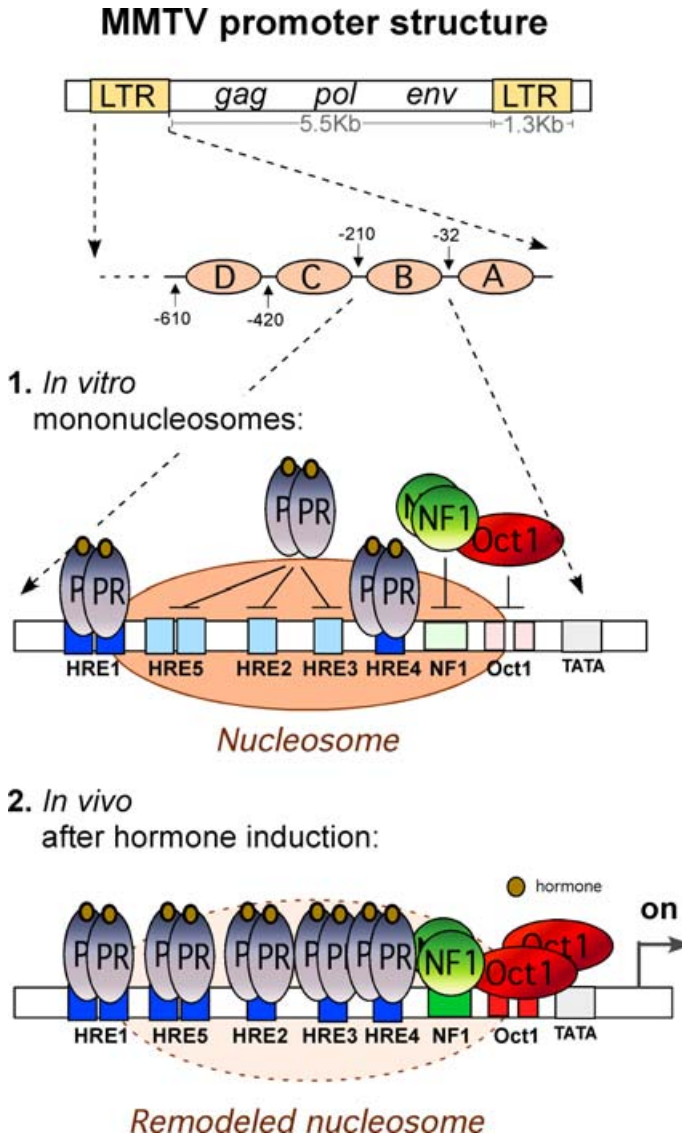

FIGURE 1. Schematic representation of the main *cis* elements in the MMTV promoter and their occupancy in nucleosomes assembled *in vitro* (1) and in intact cells after hormone induction (2). The positions covered by the main population of histone octamers are indicated by the oval labeled nucleosome or nucleosome-like particle. The various HREs, the NF1 binding site, the octamer factor 1 binding sites (Oct1), and the TATA box are indicated. The numbers refer to the distance in nucleotides from the transcription start site. The hormone receptor (PR) dimers are represented by violet ovals, the NF1 dimer by green circles, and Oct1 by red ovals. Colors appear in on-line version.

THE LESSONS FROM *IN VITRO* ASSEMBLED DYNAMIC MINICHROMOSOMES

To study the biochemistry of the interaction between hormone receptors and chromatin-organized MMTV promoter sequences, we made use of chromatin assembly systems that generate arrays of nucleosomes mimicking the behavior of natural chromatin. In our hands the best results were obtained with extracts from preblastodermic *Drosophila* embryos, which contain abundant core histones and the machinery needed for efficient chromatin assembly.[27] Minichromosomes assembled in these extracts exhibit the same translational and rotational positioning of nucleosomes over the MMTV promoter as detected in the chromatin of intact breast cancer cells, with a nucleosome occupying the HREs and the NF1 binding sites. In the absence of PR and NF1, these MMTV minichromosomes are transcriptionally silent when assayed in a cell-free transcription system. Addition of the factors individually results in a weak stimulation of transcription by PR and little or no activation by NF1. However, addition of both factors together causes a strong synergistic transcriptional activation, which is dependent on preincubation of the minichromosomes with PR in the presence of ATP, suggesting that an ATP-dependent chromatin remodeling process is needed.[28] Preliminary experiments indicated that the complex responsible for this remodeling event in embryonic extracts is NURF,[29] which is recruited to the minichromosomes by PR.[28]

In DNA footprinting experiments at high concentrations of PR, we detect ATP-dependent binding of the receptors to the HREs, while NF1 is unable to bind to the MMTV promoter in minichromosomes. However, preincubation of the minichromosomes with PR and ATP facilitates binding of NF1 to the promoter, generating a continuous footprint over the HREs and the NF1 site,[28] as reported *in vivo*.[22] Thus, in extracts that assemble dynamic chromatin, one can reproduce the physiological behavior of the MMTV promoter.

At lower, more physiological concentrations of PR, no DNA footprint is observed even in the presence of ATP, but the low levels of receptor are sufficient to synergize with NF1 and to generate a continuous footprint over the HREs and the NF1. These results indicate that under physiological conditions not only does PR help NF1 to bind, but NF1 is needed for optimal PR binding.[28] Intriguingly, the transactivation domain of NF1 is not needed for this reciprocal synergism with PR, suggesting that the only function of NF1 is to stabilize the "open" conformation of the nucleosome and thus to facilitate access of PR to the hidden HREs.

H2A/H2B DIMER DISPLACEMENT *IN VIVO* AND *IN VITRO*

To identify the ATP-dependent remodeling activity involved in opening the MMTV chromatin and to define the nature of the "open" nucleosomal

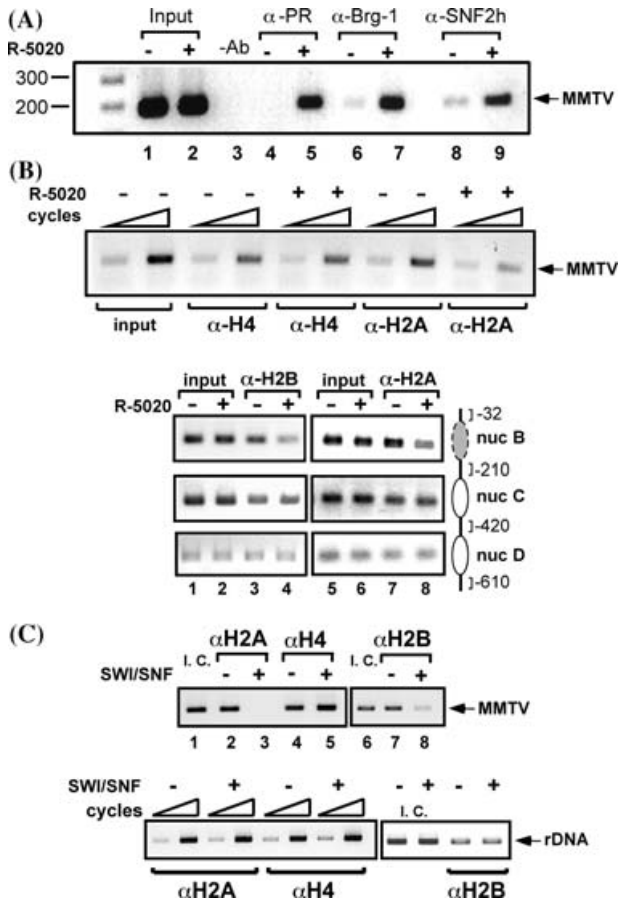

FIGURE 2. Binding of factors and histone stoichiometry on the MMTV promoter. (**A**) Chromatin immunoprecipitation (ChIp) experiments in T47D-ML cells, carrying a single copy of the MMTV promoter integrated in chromatin: recruitment of PR and ATP-dependent chromatin remodeling complexes. Cells were treated with the synthetic progestin R5020 or with vehicle (−) for 30 min and submitted to ChIp experiments using antibodies to PR, the Brg1 ATPase, or the hSnf2h ATPase. The input (1%) and the precipitated DNA were amplified by PCR using oligos specific for nucleosome B of the MMTV promoter.[30] (**B**) ChIp experiments in T47D-ML cells: displacement of H2A and H2B from the MMTV promoter following hormone induction. Cells were treated as described in (**A**) and submitted to ChIp experiments using antibodies against histones H2A, H4 (*upper panel*), and H2B (*lower panel*). The input and the precipitated DNA were amplified using probes specific for MMTV nucleosome B (*upper row*), nucleosome C (*middle row*), and nucleosome D (*bottom row*). (**C**) Displacement of histones H2A and H2B from MMTV but not from rDNA nucleosomes. Recombinant histone octamers were used to assemble nucleosomes by salt dialysis on DNA fragments of equal length derived from the MMTV promoter or from the mouse rDNA promoter. Both sequence position nucleosomes in two main translational frames are seen. On incubation with purified ySWI/SNF in the presence of ATP, ChIp experiments using antibodies against histones H2A, H2B, and H4 were performed.

conformation, we performed chromatin immunoprecipitation (ChIP) experiments in T47D breast cancer cells carrying a single copy of the MMTV promoter.[22] Thirty minutes after treatment with the synthetic progesterone analogue R5020, we could detect PR bound to the MMTV promoter, along with the coactivator Src-1 and the chromatin remodeling complexes Brg1 and SNF2h (FIG. 2 A).[30] Thus, these two remodeling ATPases could be part of the complexes responsible for the ATP-dependent changes in chromatin sensitivity to nucleases detected 30 min after hormone exposure.[22] Simultaneously, there is a selective loss of histones H2A and H2B from the promoter nucleosome B, but not from the adjacent nucleosomes C or D (FIG. 2 B).[30]

To test whether ATP-dependent remodeling complexes can displace histones H2A and H2B from MMTV nucleosomes, we performed experiments with *in vitro* assembled nucleosomes and purified ySWI/SNF complex isolated from *Saccharomyces cerevisiae*. To our surprise we found that in the presence of ATP, ySWI/SNF could displace H2A and H2B from MMTV promoter nucleosomes, but not from a mouse ribosomal promoter nucleosome assembled on DNA fragments of the same length (FIG. 2 C).[30] Since the proteins used for this assay were highly purified or recombinant, we conclude that the nucleotide sequence contains topological information that determines not only nucleosome positioning, but also the outcome of the remodeling process.

HORMONE RECEPTORS AND NF1 CAN BIND TO THE MMTV PROMOTER IN POSITIONED H3/H4 TETRAMERS

Is the displacement of both H2A/H2B dimers a reasonable model for explaining the binding of PR and NF1 to MMTV promoter chromatin on hormone induction? We know that a tetramer of histones H3 and H4 positions MMTV-promoter sequences in a very similar way as a histone octamer, and that NF1 can bind to a H3/H4 tetramer particle with relatively high affinity.[31] The question is whether PR can access the central HREs in MMTV sequences organized around an H3/H4 tetramer. To answer this question, we performed band shift experiments with free DNA as well as with DNA assembled around a histone octamer or around an H3/H4 tetramer. The results show that whereas PR could only bind to the exposed HREs on the octamer particle, it could clearly access the central HREs in the tetramer particle. Thus, a tetramer of histones H3 and H4 represents a plausible model for the structure of the "open" nucleosome conformation detected on hormone induction.

HISTONE H1 PARTICIPATES IN OPTIMIZING HORMONE INDUCTION

The experiments described so far do not take into account the linker histones, an important structural component of metazoan chromatin. Linker histones

form a large family of proteins that share a common globular domain and exhibit variable C-terminal and N-terminal extensions, with basic residues and sites for phosphorylation by various kinases. The globular domain binds DNA at the entry site and at the pseudo dyad, whereas the C-terminal domain contacts the DNA at the exit site and imposes a change on its direction. The structure of the bound N-terminal extension has not been solved. Given these interactions linker histones are considered to seal the nucleosomal DNA and therefore to limit the dynamics of the nucleosome. In fact it has been shown that in the presence of bound histone H1, the ySWI/SNF complex cannot remodel nucleosomes.[32] We therefore investigated the role of histone H1 in the hormonal induction of the MMTV promoter.

Using mononucleosomes assembled by salt dialysis, we found that histone H1 binds asymmetrically to MMTV nucleosomes, with a clear preference for the distal 5′ end of nucleosomal DNA.[33] In agreement with the accepted model, we found that incorporation of H1 into MMTV minichromosomes increases nucleosome spacing and reduces access of general transcription factors to the promoter, thus inhibiting basal transcription.[34] However, the absolute values of transcription and induction by a combination of PR and NF1 were enhanced in H1 containing minichromosomes.[34] This unexpected effect was due to the better positioning of nucleosomes in the presence of H1,[33] and, as a consequence, a better binding of PR[33,34] and a higher proportion of promoters participating in transcription.[34]

But how is the H1 obstacle overcome by the ATP-dependent remodeling activities involved in hormone induction? We found that in the presence of bound PR, H1 is phosphorylated and subsequently removed from the promoter on transcription initiation.[34] Phosphorylation of H1 allows nucleosome remodeling by ATP-dependent complexes.[32] Thus, H1, a structural component of chromatin that functions as a general repressor of transcription, contributes to a better regulation of a hormone-inducible promoter by reducing basal transcription and improving induced transcription.

NONGENOMIC EFFECTS OF PROGESTINS

Apart from their direct transcriptional effects, steroid hormones also exhibit rapid cytoplasmic effects, such as the transient activation of several kinase cascades. The ultimate targets of the activated kinase cascades are not well defined, but likely include transcription factors and factors involved in cell cycle control.[35] Traditionally the nongenomic and genomic actions of steroid hormones have been considered as two independent pathways, but we have tested the possibility that the two pathways converge in the modification of structural components of chromatin.

Five minutes after progesterone administration to breast cancer cells, there is an increase in activity of the components of the Src/Ras/Erk cascade, which

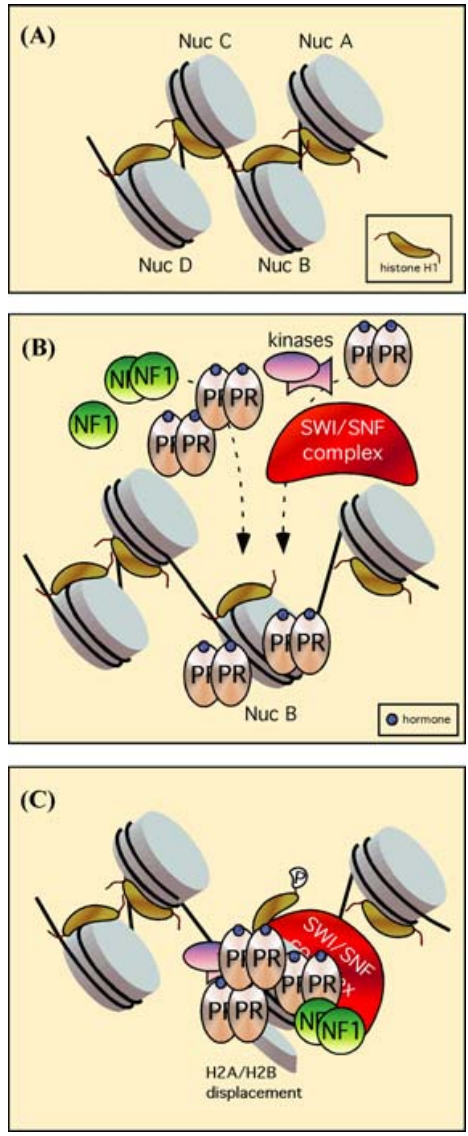

FIGURE 3. Hypothetical model for the initial steps of MMTV promoter induction. Histone H1 is associated with chromatin and improving nucleosome positioning and tightening nucleosome structure. In this way H1 contributes to a further silencing of the gene in the absence of hormone-activated PR, but improves PR binding to the exposed HRE1.[33,34] Nucleosome-bound PR recruits kinases that phosphorylate H1 and H3. Subsequently an ATP-dependent chromatin-remodeling complex is recruited to the promoter and catalyzes displacement of H2A/H2B dimers and NF1 binding. NF1 binds to its cognate site and maintains the open conformation of the nucleosome, permitting additional PR molecules to bind to the previously inaccessible HREs, enhancing recruitment of coactivators and the general transcriptional machinery. It is around this time that H1 is displaced from the promoter.

is essential for progestin-induced cell proliferation.[36] This effect is mediated by a specific interaction between two domains of the N-terminal half of PR and the ligand-binding-domain of ERα, which is activated in the absence of estrogens.[37] Activated ERα interacts directly with c-Src and activates its tyrosine kinase activity and consequently the whole MAP kinase cascade. Because some of the kinases that phosphorylate core histones (Msk1 and 2) and histone H1 (Cdk2) are downstream substrates of Erk, it is conceivable that the rapid cytoplasmic effects of steroid hormones are in some way related to their chromatin targets. Indeed, selective inhibition of Erk or Msk activation in breast cancer cells interferes with chromatin remodeling and blocks transcriptional activation of the promoter. Activated Msk is recruited to the MMTV promoter in a complex with activated PR and Erk and phosphorylates histone H3 at serine 10, leading to displacement of HP1γ and activation of chromatin remodeling.[38] Inhibiting the activation of the kinases compromises progestin activation of classical progesterone target promoter. Our results point to a hitherto unsuspected link between rapid kinase activation and gene induction by steroid hormones.

A hypothetical model of how all the processes described in this review could take place on the MMTV promoter is proposed in FIGURE 3.

In rat endometrial stromal cells, which have no ERα but only minute amounts of ERβ, progestins activate the Src/Ras/Erk pathway and the PI3K/Akt pathway via an interaction of PR with ERβ leading to induction of cell proliferation.[39] The low amount of PR in these cells precludes transcriptional activation of progesterone target genes. Moreover, picomolar concentrations of progestins unable to trigger transcriptional gene regulation are sufficient for activating cell proliferation.[39] Thus, in these cells the effects of progestins on cell proliferation are physiologically uncoupled from their genomic effects. These results indicate that the nature of the cross talk between various signaling pathways used by steroid hormones are cell type–specific and probably specified in the course of cell differentiation.

ACKNOWLEDGMENTS

We wish to thank Jofre Font, CRG, for PR preparation. G.P.V. was a recipient of a fellowship of the Ramón y Cajal Programme. The experimental work was supported by grants from the Departament d´Universitats Recerca i Societat de la Informació (DURSI), Ministerio de Educación y Ciencia (MEC) BMC 2003-02902 and Fondo de Investigación Sanitaria (FIS) PI0411605 and CP04/00087.

REFERENCES

1. EISEN, J.A., K.S. SWEDER & P.C. HANAWALT. 1995. Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. Nucleic Acids Res. **23:** 2715–2723.

2. HASSAN, A.H., P. PROCHASSON, K.E. NEELY, *et al*. 2002. Function and selectivity of bromodomains in anchoring chromatin-modifying complexes to promoter nucleosomes. Cell **111:** 369–379.
3. BOYER, L.A., R.R. LATEK & C.L. PETERSON. 2004. The SANT domain: a unique histone-tail-binding module? Nat. Rev. Mol. Cell. Biol. **5:** 158–163.
4. FLANAGAN, J.F., L.Z. MI, M. CHRUSZCZ, *et al*. 2005. Double chromodomains cooperate to recognize the methylated histone H3 tail. Nature **438:** 1181–1185.
5. JENUWEIN, T. & C.D. ALLIS. 2001. Translating the histone code. Science **293:** 1074–1080.
6. STRAHL, B.D. & C.D. ALLIS. 2000. The language of covalent histone modifications. Nature **403:** 41–45.
7. AGALIOTI, T., G. CHEN & D. THANOS. 2002. Deciphering the transcriptional histone acetylation code for a human gene. Cell **111:** 381–392.
8. JACOBSON, R.H., A.G. LADURNER, D.S. KING & R. TJIAN. 2000. Structure and function of a human TAFII250 double bromodomain module. Science **288:** 1422–1425.
9. ROBINSON-RECHAVI, M., H. ESCRIVA GARCIA & V. LAUDET. 2003. The nuclear receptor superfamily. J. Cell. Sci. **116:** 585–586.
10. EVANS, R.M. 1988. The steroid and thyroid hormone receptor superfamily. Science **240:** 889–895.
11. O'MALLEY, B.W. & O.M. CONNEELY. 1992. Orphan receptors: in search of a unifying hypothesis for activation. Mol. Endocrinol. **6:** 1359–1361.
12. ZARET, K.S. & K.R. YAMAMOTO. 1984. Reversible and persistent changes in chromatin structure accompany activation of a glucocorticoid-dependent enhancer element. Cell **38:** 29–38.
13. CHANDLER, V.L., B.A. MALER & K.R. YAMAMOTO. 1983. DNA sequences bound specifically by glucocorticoid receptor *in vitro* render a heterologous promoter hormone responsive *in vivo*. Cell **33:** 489–499.
14. PAYVAR, F., D. DEFRANCO, G.L. FIRESTONE, *et al*. 1983. Sequence-specific binding of glucocorticoid receptor to MTV DNA at sites within and upstream of the transcribed region. Cell **35:** 381–392.
15. SCHEIDEREIT, C., S. GEISSE, H.M. WESTPHAL & M. BEATO. 1983. The glucocorticoid receptor binds to defined nucleotide sequences near the promoter of mouse mammary tumour virus. Nature **304:** 749–752.
16. CHALEPAKIS, G., J. ARNEMANN, E. SLATER, *et al*. 1988. Differential gene activation by glucocorticoids and progestins through the hormone regulatory element of mouse mammary tumor virus. Cell **53:** 371–382.
17. VON DER AHE, D., S. JANICH, C. SCHEIDEREIT, *et al*. 1985. Glucocorticoid and progesterone receptors bind to the same sites in two hormonally regulated promoters. Nature **313:** 706–709.
18. CATO, A.C., D. HENDERSON & H. PONTA. 1987. The hormone response element of the mouse mammary tumour virus DNA mediates the progestin and androgen induction of transcription in the proviral long terminal repeat region. EMBO J. **6:** 363–368.
19. RICHARD-FOY, H. & G.L. HAGER. 1987. Sequence-specific positioning of nucleosomes over the steroid-inducible MMTV promoter. EMBO J. **6:** 2321–2328.
20. BRUGGEMEIER, U., L. ROGGE, E.L. WINNACKER & M. BEATO. 1990. Nuclear factor I acts as a transcription factor on the MMTV promoter but competes with steroid hormone receptors for DNA binding. EMBO J. **9:** 2233–2239.

21. KALFF, M., B. GROSS & M. BEATO. 1990. Progesterone receptor stimulates transcription of mouse mammary tumour virus in a cell-free system. Nature **344:** 360–362.
22. TRUSS, M., J. BARTSCH, A. SCHELBERT, *et al*. 1995. Hormone induces binding of receptors and transcription factors to a rearranged nucleosome on the MMTV promoter *in vivo*. EMBO J. **14:** 1737–1751.
23. BRÜGGEMEIER, U., M. KALFF, S. FRANKE, *et al*. 1991. Ubiquitous transcription factor OTF-1 mediates induction of the mouse mammary tumour virus promoter through synergistic interaction with hormone receptors. Cell **64:** 565–572.
24. PINA, B., U. BRUGGEMEIER & M. BEATO. 1990. Nucleosome positioning modulates accessibility of regulatory proteins to the mouse mammary tumor virus promoter. Cell **60:** 719–731.
25. EISFELD, K., R. CANDAU, M. TRUSS & M. BEATO. 1997. Binding of NF1 to the MMTV promoter in nucleosomes: influence of rotational phasing, translational positioning and histone H1. Nucleic Acids Res. **25:** 3733–3742.
26. BARTSCH, J., M. TRUSS, J. BODE & M. BEATO. 1996. Moderate increase in histone acetylation activates the mouse mammary tumor virus promoter and remodels its nucleosome structure. Proc. Natl. Acad. Sci. USA **93:** 10741–10746.
27. VENDITTI, P., L. DI CROCE, M. KAUER, *et al*. 1998. Assembly of MMTV promoter minichromosomes with positioned nucleosomes precludes NF1 access but not restriction enzyme cleavage. Nucleic Acids Res. **26:** 3657–3666.
28. DI CROCE, L., R. KOOP, P. VENDITTI, *et al*. 1999. Two-step synergism between the progesterone receptor and the DNA-binding domain of nuclear factor 1 on MMTV minichromosomes. Mol. Cell. **4:** 45–54.
29. TSUKIYAMA, T. & C. WU. 1995. Purification and properties of an ATP-dependent nucleosome remodeling factor. Cell **83:** 1011–1020.
30. VICENT, G.P., A.S. NACHT, C.L. SMITH, *et al*. 2004. DNA instructed displacement of histones H2A and H2B at an inducible promoter. Mol. Cell. **16:** 439–452.
31. SPANGENBERG, C., K. EISFELD, W. STUNKEL, *et al*. 1998. The mouse mammary tumour virus promoter positioned on a tetramer of histones H3 and H4 binds nuclear factor 1 and OTF1. J. Mol. Biol. **278:** 725–739.
32. HORN, P.J., L.M. CARRUTHERS, C. LOGIE, *et al*. 2002. Phosphorylation of linker histones regulates ATP-dependent chromatin remodeling enzymes. Nat. Struct. Biol. **9:** 263–267.
33. VICENT, G.P., M.J. MELIA & M. BEATO. 2002. Asymmetric binding of histone H1 stabilizes MMTV nucleosomes and the interaction of progesterone receptor with the exposed HRE. J. Mol. Biol. **324:** 501–517.
34. KOOP, R., L. DI CROCE & M. BEATO. 2003. Histone H1 enhances synergistic activation of the MMTV promoter in chromatin. EMBO J. **22:** 588–599.
35. BJORNSTROM, L. & M. SJOBERG. 2005. Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. Mol. Endocrinol. **19:** 833–842.
36. MIGLIACCIO, A., D. PICCOLO, G. CASTORIA, *et al*. 1998. Activation of the Src/p21ras/Erk pathway by progesterone receptor via cross-talk with estrogen receptor. EMBO J. **17:** 2008–2018.
37. BALLARE, C., M. UHRIG, T. BECHTOLD, *et al*. 2003. Two domains of the progesterone receptor interact with the estrogen receptor and are required for progesterone activation of the c-Src/Erk pathway in mammalian cells. Mol. Cell. Biol. **23:** 1994–2008.

38. VICENT, G.P., C. BALLARE, A.S. NACHT, *et al*. 2006. Induction of progesterone target genes requires activation of Erk and Msk kinases and phosphorylation of histone H3. Mol. Cell. In press.
39. VALLEJO, G., C. BALLARE, J.L. BARANAO, *et al*. 2005. Progestin activation of nongenomic pathways via cross talk of progesterone receptor with estrogen receptor beta induces proliferation of endometrial stromal cells. Mol. Endocrinol. **19:** 3023–3037.