SET-mediated Promoter Hypoacetylation Is a Prerequisite for Coactivation of the Estrogen-responsive pS2 Gene by PRMT1‡§

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Induction of transcription requires an ordered recruitment of coregulators and specific combinations of histone modifications at the promoter. Occurrence of histone H4 arginine (Arg) 3 methylation by protein arginine methyltransferase 1 (PRMT1) represents an early promoter event in ER (estrogen receptor)-regulated gene activation. However, its in vivo significance in ER signaling and the prerequisites for PRMT1 recruitment to promoters have not been established yet. We show here that endogenous PRMT1 is a crucial and non-redundant coactivator of ER-mediated pS2 gene induction in MCF7 cells. By investigating promoter requirements for PRMT1 recruitment we find that the patient SE translocation (SET) protein, which was reported to protect histone tails from acetylation, associates with the uninduced pS2 gene promoter and dissociates early upon estrogen treatment. Knockdown of SET or trichostatin A (TSA) treatment causes premature acetylation of H4 and abortion of H4 Arg3 methylation at the pS2 gene promoter resulting in diminished transcriptional induction. Thus, SET prevents promoter acetylation and is a prerequisite for the initial acetylation-sensitive steps of pS2 gene activation, namely PRMT1 function. Similar to pS2 we identify lactoferrin as a PRMT1-dependent and TSA-sensitive ER target gene. In contrast, we find that the C3 gene, another ER target, is activated in a PRMT1-independent manner and that SET is involved in C3 gene repression. These findings establish the existence of PRMT1-dependent and-independent ER target genes and show that proteins guarding promoter hypoacetylation, like SET, execute a key function in the coactivation process by PRMT1.

Protein arginine methyltransferases (PRMTs)² comprise a family of currently nine members (PRMT1–9) in mammals that play important roles in a variety of cellular processes, e.g.

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§ The abbreviations used are: PRMT, protein arginine methyltransferase; ChIP, chromatin immunoprecipitation; E2, β-estradiol; ER, estrogen receptor; ERE, estrogen response element; HA, hemagglutinin tag; HAT, histone acetyltransferase; SET, patient SE translocation; shRNA, short hairpin RNA; siRNA, small interfering RNA; TAF-1, template activating-factor 1 α, TAF-1β/SET, and the phosphoprotein pp32 interacts with unmodified histone tails in vitro and inhibits gene activation by keeping the tails inaccessible to HATs (histone acetyltransferases) (14). TAF-1β was originally identified as a factor that stimulates adenoviral replication (15) and was found to be identical with the patient SE translocation (SET) oncoprotein (16), hereafter designated SET. SET and pp32 have additionally been shown to associate with HDAC (histone deacetylase) activity and to repress nuclear hormone receptor (NR)-regulated transcription in reporter gene assays (14, 17).

Induction of transcription requires a highly ordered and sequential recruitment of coactivator proteins and specific combinations of histone modifications at the promoter, including arginine methylation. A cyclic assembly of such a series of coactivator complexes was illustrated for ER (estrogen receptor) target genes (18, 19). During transcriptional activation...
there is cross-talk between histone-modifying enzymes, e.g. PRMT1 and PRMT4 synergize with CBP/p300 in NR- and p53-activated gene expression (4, 20, 21). H4 Arg3 methylation by PRMT1 precedes histone acetylation in retinoic acid receptor and p53 signaling (4, 22). Consistently, PRMT1 prefers unmodified to preacetylated histones as in vitro substrates (3) and facilitates histone acetylation in vitro and in vivo (4, 23). Subsequently, H3 acetylation by CBP/p300 enables PRMT4-mediated promoter methylation (21). Although these findings suggest a chronological order of activating promoter events, in which PRMT1 is the first factor to be recruited followed by CBP/p300 and subsequently by PRMT4, it has recently been reported that during cyclic recruitment of coactivator complexes PRMT1 and PRMT4 are alternatively present and might exhibit redundant functions (19, 24).

By studying the role of PRMT1 in ER-regulated transcription we show that endogenous PRMT1 exerts a non-redundant function in pS2 expression and is required for full gene induction. We reveal that hypoacetylation of the pS2 promoter mediated by SET is a prerequisite for the acetylation-sensitive coactivation by PRMT1. Despite its role in preventing histone acetylation SET acts here as a transcriptional activator. In case of the ER target complement factor 3 (C3), which is not regulated by PRMT1, but seems to require acetylation for its transcription, SET is involved in gene repression. These findings establish the existence of PRMT1-dependent and -independent ER target genes and elucidate the molecular requirements for PRMT1 recruitment.

**EXPERIMENTAL PROCEDURES**

**Cell Line**—MCF7 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (Invitrogen) at 37 °C and 5% CO2. Before hormone treatment MCF7 cells were grown for 3–4 days in phenol red-free Dulbecco’s modified Eagle’s medium (Cambrex) supplemented with 5% charcoal-dextran-stripped fetal calf serum following addition of 200 nM 17β-estradiol (E2; Sigma) for the indicated times. For trichostatin A treatment, hormone-deprived cells were incubated in the presence of 500 nM TSA (Sigma) or solvent Me2SO for the indicated times. For trichostatin A treatment, hormone-deprived cells were incubated in the presence of 500 nM TSA (Sigma) or solvent Me2SO for the indicated times.

**Antibodies**—The following antibodies were employed: anti-PRMT1 was from Upstate Biotechnology (07-404) and Abcam (ab3768), anti-SET (KM1720), which does not recognize TAF-1a (25), anti-H4 Arg3 Me was from Abcam (ab412), anti-H4 Ac was from Upstate Biotechnology (06-866), anti-ERα (HC-20) and anti-p300 (C-20) were from Santa Cruz Biotechnologies, rabbit IgG was from Sigma, and anti-β tubulin was from Chemicon. Anti-PRMT6 was generated in rabbits against amino acids 346–362 of human PRMT6.

**Plasmids**—pBS/U6 short hairpin (sh) GFP and pBS/U6 shPRMT1 corresponding to nt 977–998 of human PRMT1 were recently described (6, 26). The HA-tag full-length SET cDNA construct was described (25).

**Short Interfering RNAs (siRNAs) and Transfections**—siRNA oligonucleotide duplexes were purchased from Dharmacon for targeting human PRMT1, human SET, and GFP, respectively. As TAF-1α and SET differ only in their small N-terminal region, siSET targets both splice variants. The siRNA sequences are (sense strand indicated): siPRMT1, 5′-CGUGUAUGGCUUC-GACAG-3′; siSET, 5′-AUAAUACAAACUCCGCCAA-3′; and siGFP, 5′-GAAGCUGACCUGCAGUU-3′. MCF7 cells were hormone-starved for 24 h, and subsequently siRNA duplexes (100 nM final concentration) were transfected with Lipofectamine 2000 (Invitrogen) under hormone-free conditions for 3 days. Afterward cells were treated without or with E2 and harvested for RNA, protein, or chromatin preparation. For transfection of plasmids into MCF7 cells, FuGENE (Roche Applied Science) was used. Stable MCF7 transfectants were selected with 2 µg/ml puromycin (Cayla) 48 h post-transfection. Individual clones were expanded in selection medium and analyzed by Western blot and RT-PCR.

**Reverse Transcription (RT)**—Total RNA from MCF7 cells was isolated using RNeasy kit (Qiagen). 2 µg of RNA were applied to RT by incubation with 0.5 µg oligo(dT)12 primer and 200 units of Moloney murine leukemia virus reverse transcriptase (Invitrogen). CDNA was analyzed by quantitative PCR (QPCR).

**Chromatin Immunoprecipitation (ChIP)**—Cells were cross-linked in the presence of 1% formaldehyde at 37 °C for 10 min and harvested after twice washing with cold phosphate-buffered saline. The cell pellet of a 145 cm2-dish was resuspended in lysis buffer I (5 mM PIPES, pH 8, 85 mM KCl, 0.5% Nonidet P-40) and incubated on ice for 20 min. Nuclei were collected by centrifugation (at 4 °C, 1200 rpm, 5 min), resuspended in 1 ml of lysis buffer II (10 mM Tris/HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 1% deoxycholate, 0.1% SDS, 1 mM EDTA), and incubated on ice for 10 min. Subsequently chromatin was fragmented by sonication seven times for 10 s on ice at 40% amplitude (Branson Sonifier W-250-D) to obtain an average fragment length of 0.5–1 kb. Following centrifugation (at 4 °C, 15,000 rpm, 15 min), 10% of chromatin was de-cross-linked and kept as input. From the remaining chromatin ~200 µg were used per immunoprecipitation reaction and subjected to preclearing with protein A/G-Sepharose (Amersham Biosciences), which beforehand was incubated at 4 °C for at least 2 h with 200 µg of bovine serum albumin and 100 µg of sheared salmon sperm DNA/1 ml of 50% bead slurries. Immunoprecipitations with 4 µg of antibody (or alternatively 5–8 µl when the Ig concentration was unknown) were performed overnight at 4 °C. Immunocomplexes were collected with 20 µl of protein A/G beads per reaction at 4 °C for 2 h. Precipitates were serially washed once with 1 ml of wash buffer I (20 mM Tris/HCl, pH 8, 150 mM NaCl, 5 mM EDTA, 0.2% SDS, 1% Triton X-100, 5% sucrose), wash buffer II (50 mM HEPES, pH 8, 500 mM NaCl, 1 mM EDTA, 0.1% deoxycholate, 1% Triton X-100), twice with wash buffer III (10 mM Tris/HCl, pH 8, 250 mM LiCl, 1 mM EDTA, 1% deoxycholate, 1% Nonidet P-40), and once with TE buffer (10 mM Tris/HCl, pH 8, 1 mM EDTA). Chromatin was eluted with 300 µl of elution buffer (0.1 M NaHCO3, 1% SDS) for 45 min at room temperature. All ChIP buffers contained a protein inhibitor mixture (Complete EDTA-free; Roche Applied Science). Cross-linking was reversed overnight at 65 °C. Eluted DNA was purified with QIAquick columns (Qiagen) and subjected either to standard PCR analysis for the presence of pS2 (region nt −159 to nt −463) and GAPDH promoter fragments using previously
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described primers (27, 28) or to QPCR analysis. ChIP assays have been repeated at least three times in independent experiments with the same results, and presented data sets show reproducible and representative results.

QPCR—Quantitative PCR was performed using SYBR Green (Bioline) and the Mx3000P real-time detection system (Stratagene). For RT-QPCR we used the following primers: human pS2, 5'-GCTTTGGGACAGAGAGA-3' (forward) and 5'-TAAAAACGTGGCTCTGGCC-3' (reverse); human GAPDH, 5'-AGGCAATGCTCATGACAC3'-3' (forward) and 5'-GCCCCATAACGCAAAATCC-3' (reverse); human C3, 5'-AGGTGGAAAGCTGACACCTGACACGTGACA-3' (forward) and 5'-TGGAAGGCTGAACTTGGACCAGTC-3' (reverse); human lactoferrin, 5'-TAAGGTGGAAACCGCTGAAC-3' (forward) and 5'-CCATTCTCAGAAATTTAGGCC-3' (reverse). All amplifications were performed in triplicates using 1 μl of cDNA per reaction. The triplicate mean values were calculated according to the ΔΔC, quantification method (29) using the GAPDH gene transcription as reference for normalization. Standard deviation was calculated from the triplicates. Error bars are accordingly indicated. Gene transcription was expressed as fold increase in mRNA level, whereas the mRNA level in uninduced control (siGFP or Me2SO-treated) cells was equated 1, and all other values were expressed relative to this. The represented RT-QPCR assays were reproduced at least three times in independent experiments with the same results and representative data sets are shown.

For ChIP QPCR we used the following human pS2 primer: 5'-GTGTGGCAGCAGCCTTTT-3' (reverse) and 5'-AGAGATGTCTGGAGCACAGCAGAC-3' (forward). All amplifications were performed in triplicates using 2 μl of immunoprecipitated chromatin per reaction. Mean values were normalized for the amount of input chromatin and the IgG control immunoprecipitations and expressed as fold increase in promoter association, whereas the enrichment in uninduced control cells was equated 1.

RESULTS

Functional Importance of Endogenous PRMT1 in ER Signaling—To assess the functional importance of PRMT1 in ER-regulated transcription of the pS2 gene, we used siRNA to specifically knock down PRMT1 expression in MCF7 cells, an estrogen-responsive breast cancer cell line. Cells were seeded in hormone-free medium and were subsequently transfected with siRNA. Western blot analysis of uninduced MCF7 cells identified in the siPRMT1-treated cells, and furthermore it was shown that transcript levels of GAPDH and ERα remained unaffected by E2 or siPRMT1 (supplemental Fig. 1). We obtained identical results using the recently published pBS/U6 shPRMT1 vector system (6), which targets a different region of the PRMT1 mRNA (Fig. 5A). These findings demonstrate that endogenous PRMT1 is a non-redundant coactivator of ER-mediated pS2 gene activation and is required for full gene induction.

To investigate whether this transcriptional effect in PRMT1 knockdown cells correlates with loss of H4 Arg3 methylation at the pS2 gene promoter, we performed ChIP analysis of siPRMT1-treated and control cells following E2 induction. Chromatin was subjected to immunoprecipitation with antibodies against ERα, PRMT1, Arg3-methylated H4, and control IgG. We analyzed the precipitated chromatin by standard PCR for the presence of pS2 promoter fragments including the ERE (estrogen response element) (28). Promoter occupancy of ERα, PRMT1, and Arg3-methylated H4 was hardly detectable in uninduced cells but increased 15 min post-induction and remained constant at the 30-min time point in control cells (Fig. 1C) in agreement with previous studies (18, 19). These events coincided with transcriptional activation (Fig. 1B). As expected, siPRMT1 transfection resulted in a decrease of E2-induced PRMT1 recruitment and strongly reduced levels of H4 Arg3 methylation 15 and 30 min post-induction (Fig. 1C). Binding of ERα was not affected by PRMT1 knockdown. These results show that PRMT1 is the major H4 Arg3 methyltransferase at the E2-induced pS2 promoter and that loss of H4 Arg3 methylation coincides with alleviated gene induction.

Association of Endogenous SET with the Uninduced pS2 Gene Promoter—As H4 Arg3 methylation is attenuated by histone acetylation in vitro (3), we reasoned that proteins, which pre-
vent premature hyperacetylation of histones, could be a prerequisite for early acetylation-sensitive steps of ER-mediated gene activation, like PRMT1 recruitment and activity. INHAT subunits protect histone tails from HATs and associate with HDAC activity (30). The SET protein was recently shown to interact with the unliganded ERα and to release upon hormone binding (17). In agreement with previous observations (17) we found endogenous TAF-Iα weakly expressed on transcript and protein level in MCF7 cells, whereas the splice variant β (SET) was strongly expressed (data not shown). Thus, in the following we focused on SET and investigated whether the endogenous protein associates with the pS2 gene promoter by ChIP analysis. Cells were kept in hormone-free conditions and subsequently left either untreated or stimulated for 60 min with E2. Chromatin was precipitated with anti-ERα, anti-SET, or without IgG (control) and analyzed by standard PCR for the presence of GAPDH (27) and pS2 promoter fragment. SET was bound to the uninduced pS2 promoter and became undetectable upon hormone induction (Fig. 2A). As expected, ERα weakly associated with the uninduced pS2 promoter and was recruited following addition of E2. These changes were specific for pS2 upon E2 treatment, since no enrichment of the active, non-hormone-responsive GAPDH promoter was observed.

To elucidate the kinetics of this SET displacement, we performed ChIP analysis of a shorter E2 time course. Chromatin was precipitated with antibodies against ERα, SET, PRMT1, p300, and control IgG. Standard PCR analysis of the pS2 promoter revealed recruitment of ERα and PRMT1 within 15 min post-induction, whereas SET detached from the pS2 promoter within this time (Fig. 2B). Promoter association of p300 was weak at the uninduced pS2 promoter and gradually enhanced throughout the time course (Fig. 2B). This is consistent with the view that PRMT1 precedes p300 recruitment and activity. Together these results indicate that endogenous SET associates with the uninduced pS2 promoter and detaches early after hormone induction.

The Role of SET in Transcriptional Activation of pS2—To further clarify the role of SET in pS2 gene transcription, we depleted its endogenous expression by siRNA transfection in MCF7 cells, as shown on protein level in Fig. 3A. pS2 transcription in SET knockdown and control cells was analyzed by RT-QPCR. SET reduction lowered the basal pS2 transcript level and abolished E2-induced pS2 transcription (Fig. 3B). Depletion of the SET transcript in this E2 time course was verified (supplemental Fig. 2). Transcript levels of ERα and GAPDH remained unaffected (supplemental Fig. 2). MCF7 cells stably transfected with HA (hemagglutinin)-tag SET (Fig. 3C) revealed an enhanced E2-induced pS2 transcription (Fig. 3D). These results suggest that SET contributes to transcriptional activation of pS2.

To study the function of SET at the pS2 promoter in more detail we performed ChIP assays of siGFP and siSET treated cells using antibodies against ERα, SET, Arg3-methylated H4 and acetylated H4. As demonstrated by QPCR, SET was bound to the pS2 promoter in the absence of E2 and detached within 15 min of induction in control cells. In contrast, its promoter association was greatly reduced in SET knockdown cells (Fig. 3E). Promoter binding of ERα was not affected by SET knockdown, neither in uninduced cells nor after 15 min of E2 induction (Fig. 3F). SET depletion caused increased promoter acetylation in both uninduced and induced cells, and the appearance of H4 Arg3 methylation after 15 min of E2 exposure was impeded (Fig. 3F). In control cells H4 acetylation became strongly induced upon E2 stimulation. However, the level of H4 acetylation caused by SET knockdown was not further enhanced upon E2 induction, indicating that activation events subsequent to H4 Arg3 methylation were inhibited as well. These data imply that SET keeps the pS2 promoter hypoacetylated and allows an early acetylation-sensitive step of gene activation, namely H4 Arg3 methylation.

Effect of TSA Treatment and Promoter Hyperacetylation on pS2 Gene Expression—to uncover whether premature hyperacetylation of the promoter could be responsible for delayed pS2 gene induction, we treated hormone-starved MCF7 cells with the HDAC inhibitor TSA. As recent studies reported that longer exposure to TSA inhibits ERα expression in MCF7 cells (31, 32), we determined the ER expression levels throughout 10 h of TSA treatment by Western blot analysis. We detected no decline of ER protein level within 6 h of TSA treatment (Fig. 4A). Therefore we treated cells for 2 h with TSA before adding E2 and performed RT-QPCR and ChIP analysis. TSA efficiently blocked basal and E2-induced pS2 transcription (Fig. 4B) and generated histone H4 hyperacetylation of the uninduced and weakly also of the induced pS2 promoter (Fig. 4C). However, under these conditions TSA did not influence the transcript levels of other genes, like ERα or GAPDH (supplemental Fig. 3). These data suggest that initial hypoacetylation of the pS2 promoter is important for gene activation.
Further we determined whether TSA-induced, artifical histone hyperacetylation at the promoter results in diminished H4 Arg3 methylation. In ChIP analysis E2-induced PRMT1 binding and H4 Arg3 methylation were completely abolished in TSA-treated cells (Fig. 4, C and D). Recruitment of ERα to the pS2 promoter was slightly delayed at the 15 min time point by TSA and more clearly at the 30 min time point (Fig. 4, C and D). Association of SET with the uninduced pS2 promoter was unaffected by TSA induced hyperacetylation (Fig. 4C). Likewise its dissociation from the promoter occurred with similar kinetics in control and TSA-treated cells. Together these results suggest that maintenance of a hypoacetylated state at the pS2 gene promoter is a prerequisite for H4 Arg3 methylation, an initial and crucial histone mark in pS2 activation.

Roles of PRMT1 and SET in Transcriptional Activation of Other ER Target Genes—Finally we investigated how the expression of other ER target genes is regulated by PRMT1 and SET. Therefore we checked a selection of ER-regulated genes, which contain a palindromic ERE (33, 34) for their hormone response within a 6 h time course. In addition to pS2, transcription of lactoferrin was clearly elevated after 1–2 h of E2 treatment, whereas C3 expression was weakly induced after 4–6 h (supplemental Fig. 4). In the following we analyzed C3, lactoferrin and pS2 gene expression in cells either stably expressing shPRMT1 (Fig. 5A) or treated with TSA (Fig. 5B) and siSET (Fig. 5C) by QPCR, respectively.

 Knockdown of PRMT1 was established with the pBS/U6 shPRMT1 vector system (6) and verified (supplemental Fig. 5). MCF7 transfectants generated with the recently published pBS/U6 shGFP construct (26) served as control. PRMT1 depletion did not influence C3 gene activation, i.e. the C3 transcript level increased in shGFP and shPRMT1 cell lines after 6 h of E2 treatment (Fig. 5A). In contrast, transcription of lactoferrin resembled that of pS2 (Fig. 5A), since reduced levels of PRMT1 resulted in a lower basal lactoferrin expression and delayed induction. In the following we analyzed effects of TSA treatment on gene expression. We restricted the TSA treatment to 6 h (in case of C3 up to 8 h), i.e. 2 h pretreatment with TSA and subsequently addition of E2 until 4 h (in case of C3 until 6 h). Induction of lactoferrin expression was diminished in the presence of TSA similar to its effect on pS2 (Fig. 5B). In contrast, transcription of the C3 gene was increased by TSA (Fig. 5A). As expected, SET knockdown resulted in a delayed pS2 gene induction, but had the inverse effect on C3 expression leading to increased transcription (Fig. 5C). SET depletion did not influence lactoferrin expression (Fig. 5C).

Collectively these results suggest that the C3 gene differs in its regulation from lactoferrin and pS2, because its transcrip-
tion is induced PRMT1-independently and premature hyperacetylation, either triggered by TSA treatment or SET knockdown, seems to cause enhanced C3 transcription. Here, SET acts as a repressor of transcription. In contrast transcription of both pS2 and lactoferrin depend on PRMT1. Therefore proteins maintaining promoter hypoacetylation, e.g. SET or equivalent proteins, act here as transcriptional activators and enable the initial acetylation-sensitive coactivation by PRMT1.

**DISCUSSION**

A plethora of NR coactivator proteins, including many histone modifying enzymes, have been identified, which act in a coordinated and interdependent manner to prepare promoters for transcription. PRMT recruitment and histone arginine methylation belong to the well established coactivation events of ligand-activated NRs (2, 3, 9, 35). Occurrence of PRMT1 at promoters represents an early step in gene activation and induces a transcriptional precommitted chromatin state (22). However, not all NR target genes require PRMT1 for their transcriptional activation, as demonstrated for the thyroid hormone receptor (36).

In this study we investigated the role of PRMT1 in ER-mediated pS2 gene activation. Although the enzyme has been reported to associate with the pS2 promoter following hormone induction, its significance in NR signaling has not been established. Recent reports even suggested functional redundancy between PRMT1 and PRMT4 in the cyclic coactivator complexes (19, 24). Knockdown by RNA interference using two alternative siRNA sequences against the PRMT1 transcript revealed its importance for E2-induced pS2 activation in vivo. This effect is likely attributable to its enzymatic activity, since we found that depletion of PRMT1 resulted in a loss of H4 Arg3 methylation at the pS2 promoter. The basal pS2 mRNA level was also reduced by PRMT1 knockdown. This might be due to some residual estrogen in the hormone-starved cell culture, which contributes to pS2 gene activity under uninduced conditions.

Given that H4 Arg3 methylation primes promoters for transcription and precedes histone acetylation and other modifications, we aimed at characterizing early promoter events, that promote PRMT1 recruitment. We speculated that SET, which is the TAF-I isoform primarily expressed in MCF7 cells (17), could be such a prerequisite. SET is part of the INHAT complex, which binds to unmodified histone tails and keeps them inaccessible to HATs (14). Since overexpressed INHAT subunits are able to repress NR-regulated reporter genes and associate with HDAC activity, it was suggested that the complex is involved in transcriptional repression (14, 17). Recent work showed that SET also binds to the transcription factor KLF5 antagonising p300-mediated acetylation and activation of KLF5 (37). Furthermore, SET participates in gene silencing by inhibiting DNA demethylation (38). In contrast, other reports suggest that SET contributes to gene activation (39–41).

In ChIP assays SET associated with the pS2 promoter before hormone treatment and detached shortly after addition of E2.
Although this promoter occupancy suggests a repressive function of SET, knockdown and overexpression experiments revealed that it acts as an activator of pS2 transcription. SET depletion resulted in a premature acetylation of histone H4 in uninduced cells and subsequent loss of H4 Arg3 methylation. Consistently, it was reported that PRMT1 prefers non-acetylated histones as substrate (3). Furthermore, we show that premature H4 acetylation caused by SET knockdown was not further enhanced upon E2 induction, indicating that activating events following H4 Arg3 methylation were also abolished. This is in agreement with previous observations that H4 Arg3 methylation stimulates recruitment of HATs and histone acetylation (3, 22, 23, 35). Our findings reveal that SET maintains a hypoacetylated p52 promoter state before PRMT1 recruitment and allows the ordered establishment of activating histone marks, as illustrated in Fig. 6.

Our TSA experiments strengthen this view, since TSA-induced promoter hyperacetylation resulted in inhibition of pS2 gene induction and coincided with suppression of E2-mediated PRMT1 recruitment and H4 Arg3 methylation. TSA additionally delayed the E2-induced promoter association of ER. As we excluded that TSA impaired ER expression at the chosen conditions, the alleviated ER recruitment might be due to lack of proper chromatin remodeling and hence a less stable promoter association of ER. It has been demonstrated that binding of PRMT1 to ER target genes is mediated by liganded ER and p160 coactivator as a bridging factor (42). However, we reason that the observed delay in ER recruitment unlikely accounts for the abrogation of PRMT1 function in the TSA experiment, in particular since ER binding was reproducibly hardly influenced by TSA at the 15-min time point. A similar inhibitory effect of TSA on H4 Arg3 methylation was observed during myeloid cell differentiation (22). TSA affected pS2 transcription and promoter hyperacetylation much stronger compared with the effects of SET knockdown. This implies the existence of additional proteins, which protect the pS2 promoter against premature hyperacetylation but might also be due to a permanently impaired ER recruitment.

Recent data demonstrated that modifications, like acetylation and phosphorylation, prevent in vitro interaction of SET and other INHAT subunits with histone N termini (14, 30, 43). These observations suggest that histone modifications might trigger SET displacement from the promoter. In contrast, our results show that in vivo dissociation of SET seems to occur independently of histone H4 acetylation. SET was previously shown to interact with the unliganded ERα (17), that weakly associates with the pS2 promoter. As the interaction between SET and ERα is hormone-sensitive, SET displacement from the promoter within 15 min of hormone induction is likely due to its lost interaction with ERα.

In an attempt to investigate the implication of PRMT1 and SET on other ER target genes, we identified the C3 gene, which

![Figure 5](http://www.jbc.org/)
in contrast to pS2 is transcribed PRMT1-independently and derepressed by TSA treatment or SET knockdown. These results suggest that SET fulfills a dual function in ER-regulated transcription and behaves as a transcriptional activator and repressor in a gene specific manner. ER target genes, like pS2, which depend on coactivation by PRMT1, require inhibition of premature promoter acetylation for their transcriptional activation. In contrast, C3, which is not coactivated by PRMT1, can be enhanced or repressed by TSA treatment (22, 47). Distinct NR-regulated genes require different sets of coactivators and different combinations of histone marks for initiation of transcription. It was recently reported that ERE sequences modulate the conformation of bound ER and hence influence its interaction with coregulatory proteins (34, 48). This causes a gene-specific order of coactivator recruitment and histone modifications at the promoter. PRMT1 might be an important regulator of such gene-specific activation patterns.

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