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Inhibition of the 26S proteasome blocks progesterone receptor-dependent transcription through failed recruitment of RNA polymerase II

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Abstract

In the present study, we investigated the involvement of protein degradation via the 26S proteasome during progesterone receptor (PR)-mediated transcription in T-47D cells containing a stably integrated MMTV–CAT reporter construct (CAT0 cells). Progesterone induced CAT and HSD11β2 transcription while co-treatment with the proteasome inhibitor, MG132, blocked PR-induced transcription in a time-dependent fashion. MG132 treatment also inhibited transcription of β-actin and cyclophilin, but not two proteasome subunit genes, PSMA1 and PSMC1, indicating that proteasome inhibition affects a subset of RNA polymerase II ($RNAP_{II}$)-regulated genes. Progesterone-mediated recruitment of $RNAP_{II}$ was blocked by MG132 treatment at time points later than 1 h that was not dependent on the continued presence of PR, associated cofactors, and components of the general transcription machinery, supporting the concept that proteasome-mediated degradation is needed for continued transcription. Surprisingly, progesterone-mediated acetylation of histone H4 was inhibited by MG132 with the concomitant recruitment of HDAC3, NCoR, and SMRT. We demonstrate that the steady-state protein levels of SMRT and NCoR are higher in the presence of MG132 in CAT0 cells, consistent with other reports that SMRT and NCoR are targets of the 26S proteasome. However, inhibition of histone deacetylation by trichostatin A (TSA) treatment or SMRT/NCoR knockdown by siRNA did not restore MG132-inhibited progesterone-dependent transcription. Therefore, events other than histone deacetylation and stability of SMRT and NCoR must also play a role in inhibition of PR-mediated transcription.

Keywords: Transcription; Progesterone receptor; MMTV; HSD11β2; 26S proteasome; Degradation; MG132; Inhibition; Coactivators; $RNAP_{II}$; General transcription machinery; Corepressors; Histone acetylation; Histone deacetylase; Trichostatin A; CAT0; HeLa; Chloramphenicol acetyltransferase; Luciferase

1. Introduction

The nuclear receptor (NR) superfamily is the largest known group of eukaryotic transcription factors and includes receptors for the steroids, retinoids, thyroid and Vitamin D hormones, and orphan receptors for which no ligand has been identified [1]. All members of the NR superfamily share common structural domains labeled A through F, that contain regions for DNA and ligand binding, dimerization, nuclear localization, and heat-shock protein interaction. The N- and C-terminal portions of the NR contain two activation functions, AF-1 and AF-2, respectively, critical for interacting with cofactors (coactivators and corepressors) that modulate the transcriptional response.

To date, many coactivators have been identified, each belonging to a group that supports formation of the pre-initiation complex by re-organizing the chromatin to facilitate entry of or directly recruit the general transcription factors (GTFs). The steroid receptor coactivators (SRCs) [2], CREB-binding protein (CBP) [3], p300 [4], and PCAF (p300/CBP-associated cofactor) [5] proteins each possess histone acetyltransferase activity that neutralizes the positive charge of lysines within histones by attaching an acetyl group to the ϵ-amine group. Members of the SWI/SNF (switch/sucrose non-fermenting) family are ATP-dependent chromatin remodelers that slide nucleosomes in *cis* or relax the DNA–nucleosome interaction to facilitate entry of

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transcription factors [6,7]. Thyroid receptor-associated protein (TRAP)/mediator complexes represent a third group of coactivators capable of bridging NRs to GTFs at the promoter [8].

A critical problem exists in coordinating recruitment of these coactivators to ligand-activated NRs since many of these share an "NR box" motif important for mediating the coactivator–NR interaction, indicating that simultaneous binding of multiple coactivators to NRs could not exist [2]. Additionally, the order of addition of coactivators seems to be important since recent data suggest that a sequential model of coactivator binding is critical for gene activation [9–12]. Thus, mechanisms must exist that (1) control the order of addition of coactivators, and (2) provide for the dynamic exchange of coactivators and other transcription components.

The identification of ubiquitin conjugating [13] and ubiquitin ligase [14,15] enzymes that play a role in nuclear receptor transactivation hints that the ubiquitin-degradation pathway may coordinate coactivator exchange by ubiquitylating and destroying the coactivator once its role in transcription (i.e. histone acetylation, chromatin remodeling, GTF recruitment) is accomplished [16]. In support of this possibility, several coactivators, including the SRC family, p300, and CBP, have been recently shown to be targeted for destruction by the proteasome. In addition to its histone acetylation activity, p300 also has been shown to possess ubiquitin ligase activity and cooperates with MDM2 (minute double mutant) to promote ubiquitylation of p53 [17,18]. Perhaps the most significant evidence that implicates the link between NR-dependent transcription and degradation came from our lab demonstrating that proteasome-mediated degradation of the estrogen receptor α (ERα) is required for its transactivation [19]. Importantly, similar observations have been reported for other NRs including, progesterone receptor (PR) [20], androgen receptor (AR) [21], thyroid hormone receptor (TR) [22], and retinoic acid receptor α (RARα) [23], indicating that proteasome involvement during transcription is broader than initially suspected. However, not all NR-regulated genes require this degradation function, since it is known that proteasome inhibition enhances the transcriptional activity of glucocorticoid receptor (GR) [24,25]. Reasons for this are not fully understood, but recent evidence suggests that GR remains bound to the promoter longer in the presence of proteasome inhibition, which is consistent with a higher transcription output [26].

In order to rigorously test the involvement of the proteasome during transcription we took advantage of a T-47D (CAT0) cell line that expresses both forms of the progesterone receptor (PRA and PRB) and contains a stably integrated MMTV–CAT (mouse mammary tumor virus fused upstream to chloramphenicol acetyltransferase) chimeric reporter construct that PR is capable of activating. This system allows us to investigate the assembly of transcription factors on the MMTV promoter and production of CAT mRNA transcripts during proteasome inhibition. An earlier report demonstrated that proteasome inhibition prevents recruitment of phosphorylated $RNAP_{II}$ to the estrogen-responsive pS2 promoter [27]. However, this study utilized cells that were pre-treated for 6 h with proteasome inhibitor before adding estradiol. In the present study, we simultaneously treated CAT0 cells with progesterone and proteasome inhibitor, and demonstrate that proteasome function is necessary to recruit $RNAP_{II}$ to the MMTV promoter during late, but not early, progesterone-mediated transcription. This observation is concomitant with untimely formation of a repressive chromatin structure at the promoter, mediated, in part, through stabilization of the corepressors, NCoR and SMRT [13,28]. However, inhibition of histone deacetylase (HDAC) activity or knockdown of NCoR and SMRT is insufficient in restoring recruitment of $RNAP_{II}$, suggesting that additional proteasome-mediated events play a role in sustained PR-mediated transcription.

2. Materials and methods

2.1. Reagents

Progesterone, trichostatin A (TSA), DMSO, MG132, anti-mouse β-actin antibody, and protease inhibitor cocktail used in the experiments were purchased from Sigma. The antibodies against PR (H-190), RAC-3 (C-20), CBP (A-22), $TAF_{II}250$ (6B3), TFIIB (C-18), and HDAC3 (H-99) were purchased from Santa Cruz. The antibodies against total $RNAP_{II}$ (8WG16) and phosphoserine-2 within the CTD of $RNAP_{II}$ (H5) were purchased from Covance. The antibodies against p300 (05-257), histone H4 acetyl-lysine 5 (06-759), and histone H4 acetyl-lysine 8 (06-760) were purchased from Upstate Biotechnology. The antibodies against SMRT, NCoR, and TRAP220 were a gift from J. Wong (Baylor College of Medicine, Houston, TX). The antibody against Skip1 was a gift from D. Dowhan (Baylor College of Medicine, Houston, TX).

2.2. Mammalian cell culture conditions

The T-47D CAT0 cell line was provided and used with permission from Jack-Michel Renoir (Centre Nationale de la Recherche Scientifique, Chatenay-Malabry, France). The T47D-CAT0 cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 8% fetal bovine serum (Invitrogen) plus 200 μg/mL of geneticin in a 37 °C incubator containing a 5% CO_2 environment. HeLa cells were maintained in a similar environment and medium (without geneticin).

2.3. Chromatin immunoprecipitation

T-47D CAT0 cells were grown to 95% confluence in DMEM supplemented with 5% charcoal–dextran stripped fetal bovine serum for 48 h, at which time progesterone, TSA, DMSO, or MG132 were added for the indicated times in the figure legends. The cells were washed twice with PBS and