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Vitamin D receptor (VDR) promoter targeting through a novel chromatin remodeling complex☆

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Abstract

We have purified nuclear complexes for Vitamin D receptor (VDR), and identified one of them as a novel ATP-dependent chromatine remodeling containing Williams syndrome transcription factor (WSTF), that is supposed to be responsible for Williams syndrome. This complex (WSTF including nucleosome assembly complex (WINAC)) exhibited an ATP-dependent chromatin remodeling activity in vitro. Transient expression assays revealed that WINAC potentiates ligand-induced function of VDR in gene activation and repression. Thus, this study describes a molecular basis of the VDR function on chromosomal DNA through chromatine remodeling.

Keywords: Vitamin D receptor; WINAC; Histone acetyltransferase; Chromatin remodeling

1. Transcriptional controls by Vitamin D receptor (VDR) requires chromatin remodeling

VDR is a member of the nuclear receptor (NR) gene superfamily, and acts as a ligand-inducible transcriptional factor [1]. At transcriptional initiation sites in the VDR target promoters, distinct classes of multiprotein complexes are thought to be indispensable for controlling transcriptions of VDR through chromatin remodeling [2,3]. These complexes modify the chromatin configuration in a highly regulated manner, like nucleosome rearrangement, and bridge the functions between regulators and basal transcription factors, along with RNA polymerase II. Two major classes of chromatin-modifying complexes have been well characterized and their anchoring to the promoters presumably requires enzyme-catalyzed modifications of histone tails in chromatin [4]. One class contains several discrete subfamilies of transcription co-regulatory complexes with either histone acetylase (HAT) or histone deacetylase (HDAC) activities to covalently modify histones through acetylation. In ligand-induced transactivation processes of nuclear receptors like VDR, the complexes containing HDAC first act to co-repress transactivation of unliganded NRs, while upon ligand binding, two HAT complexes, p160/CBP and TRRAP/GCN5, co-activate the NR function, like the other non-HAT DRIP/TRAP/SMCC co-activator complexes [5–7].

Another class of complexes are chromatin remodeling complexes to use ATP hydrolysis to rearrange nucleosomal arrays in a noncovalent manner, and make chromosomal DNA accessible for sequence-specific regulators like VDR [4]. These ATP-dependent chromatin-remodeling complexes act on transcription, DNA repair, and DNA replication as well. These complexes are further classified into subfamilies based on the major catalytic components, ATPases (SWI2/SNF2, ISWI, and Mi-2) [8,9]. Indeed, recently ligand-induced transactivation function of VDR in vitro has been shown to require a SWI2/SNF2-type chromatin remodeling complex containing pBAF180.

2. Identification of a novel Williams syndrome transcription factor (WSTF)-containing nuclear complex associate with VDR

To identify a novel co-regulator complex for VDR, HeLa cell nuclear extracts were incubated with a chimeric VDR-DEF region protein (VDR-DEF) fused to glutathione-*S*-transferase (GST), in the presence or absence of 1α,25(OH) 2D3. Mass spectrometry and the apparent molecular weights of the different proteins associated with the VDR-DEF in a ligand-dependent way led to the identification of several known components of the DRIP/TRAP/SMCC complex,

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in agreement with previous observations [5,7]. One of the ligand-independent VDR-specific interactants turned out the WSTF/WBSCR9/BAZ1B [10,11]. WSTF has been supposed to be a candidate responsible for the diverse WS disorders [10,12]. This possibility is raised by the fact that WSTF is highly homologous to hACF1 as one of the WAC family proteins [11]. Also, hACF1 is a partner of hSNF2h (a *Drosophila* ISWI homologue) to form well-characterized ISWI-based chromatin remodeling complexes [13].

To purify a WSTF-containing complex, we established a MCF7 stable transformant overexpressing FLAG-tagged WSTF. With the nuclear extracts of the stable transformants, WSTF containing complexes were isolated by multi-step purification using the GST-VDR column and an anti-FLAG affinity resin column. On the glycerol density gradient, the purified complexes with a molecular weight of greater than 670 kDa bound to the GST-VDR column and these large molecular weight fractions contained WSTF. With the mass fingerprinting, we identified all the components of the purified complex containing WSTF, and designated this complex as WSTF including nucleosome assembly complex (WINAC) [14]. WINAC consists of at least 13 components, but unexpectedly contains neither hSNF2h nor the components of known ISWI-based complexes (Table 1). Rather, the SWI/SNF type ATPases (Brg1 and hBrm) and several BAF components share with the SWI2/SNF2-based complexes [2]. Interestingly, WINAC appears to harbor three components (TopoIIb, FACTp140, and CAF-1p150) [15–17], which have not yet been found in any known ATP-dependent chromatin remodeling complexes.

3. INAC rearranges the nucleosome array around VDRE through ATP-dependent chromatine remodeling in vitro

By a standard micrococcal nuclease assay, an ATP-dependent chromatin assembly reaction was clearly induced by WINAC, indicating that Brg1/hBrm in WINAC serves as an ATPase for this ATP-dependent chromatin remodeling process. We examined the ability of WINAC to disrupt nucleosome arrays through VDR bound DNA since the known ATP-dependent chromatin remodeling complexes are potent to recognize the nucleosomal array around the binding sites of a sequence-specific regulator [18,19]. By Southern blot analysis with a pair of oligonucleotides complementary to a region in the vicinity (Promoter Probe) or to a site about 900 bp upstream (Distal Probe) of the GAL4 DBD binding sites for a chimeric VDR-DEF protein (GAL-VDR), disruption of the nucleosome arrays in the GAL4 binding site vicinity was induced only when both VDR and WINAC were present (Fig. 1A), while the other regions appeared unaffected in the nucleosome arrays. Reflecting the VDR-specific nucleosome disruption by WINAC among tested receptors (Fig. 1B), ligand-induced transactivation in vitro was potentiated by WINAC only for VDR, but for neither ERa nor PPARg (data not shown).

4. WSTF potentiated ligand-induced functions of VDR in gene induction and repression

In a transient expression analysis, 1α,25(OH)2D3 (10^{-9} M) was effective to induce VDR AF-2 transactivation function and WSTF co-activated this ligand-induced AF-2 function of VDR, but not ERα (Fig. 2A). Both Brg1 and hBrm were also effective to enhance the transactivation functions of VDR and ERα (Fig. 2A) as previously reported [20,21]. Interestingly, such co-activator-like activity of WSTF was selective for VDR, and not detected for ERa, even in the presence of Brg1/hBrm (Fig. 2A).

ChIP analysis revealed that VDR and the WINAC components were constitutively associated with the promoter irrespective of ligand binding. In the contrast, ligand-induced occupancy in the promoter was seen in TRAP220 and TIF2 with ligand-induced histone H4 acetylation though the

Table 1
Major ATP-dependent chromatin remodeling complexes

Complex name	BAF	PBAF	WINAC	hCHRAC	hACF	NoRC	xWICH
Complex size (kDa)	~2,000	~2,000	~2,000	~600	~600	~600	~600
ATPase subunit	Brgl, Brm	Brgl	Brgl, Brm	hSNF2h	hSNF2h	hSNF2h	xISWI
Chromatin remodeling			WSTF	hACF1	hACF1	TIP5	WSTF
	BAF250		BAF250				
		BAF180					
	BAF170	BAF170	BAF170				
	BAF155	BAF155	BAF155				
	BAF60a	BAF60a	BAF60a				
	BAF57	BAF57	BAF57				
	BAF53	BAF53	BAF53				
	lni1	lni1	lni1				
				p17		p80	
				p15		p50	
DNA replication			Topollβ CAF-1 p150				
Transcription elongation			FACT p140				

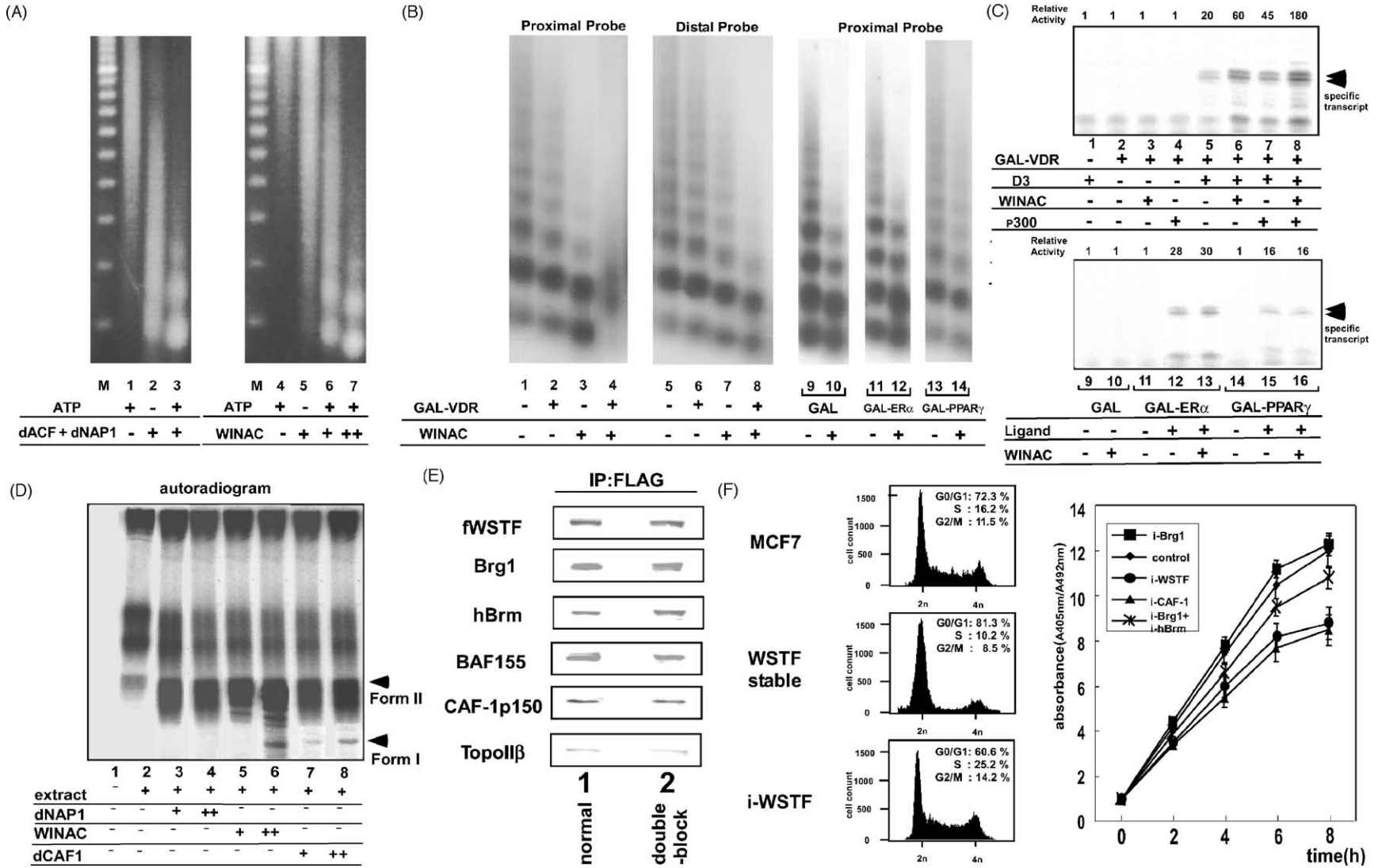
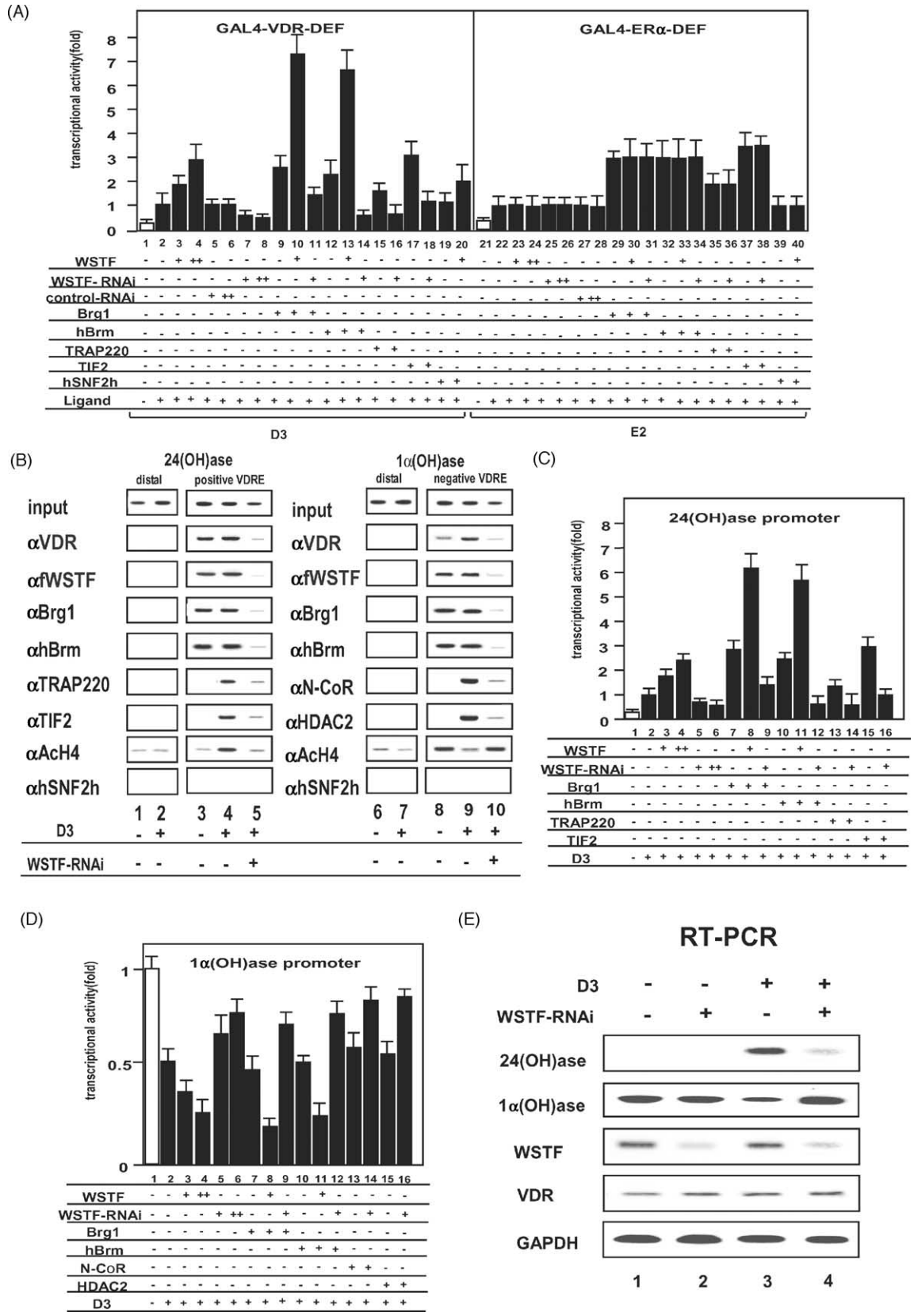


Fig. 1. WINAC as an ATP-dependent chromatin remodeling complex. (A) Chromatin reconstitution activity of WINAC. The reacted samples were subjected to partial micrococcal nuclease digestion. The molecular mass marker (M) is the 200 bp ladder. (B) Chromatin disruption by WINAC is specifically VDR dependent. Oligonucleotide probe corresponds to either a sequence between the GAL4 sites and the RNA start site (proximal probe) or 900 bp upstream of the start site (Distal Probe). (C) Potentiation of VDR transactivation by WINAC in vitro. Arrows indicate specific transcripts by transcription reactions by GAL4 derivatives. A representative result is displayed, and relative activities were calculated from three independent assays with PGI-0 vector as an internal control. (D) WINAC functions as a chromatin reconstitution factor during DNA replication in vitro. During DNA replication induced by SV40 T antigen in vitro, WINAC could form chromatin with negatively supercoiled DNA. Form I: a perfect supercoiled DNA. Form II: a relaxed form. (E) WINAC formation is unchanged in S phase. MCF7 stable transformants were cultured under either normal conditions or double thymidine block treatments. (F) Modulation of the cell cycle by altered WSTF expression. Left panel: DNA histogram of the MCF7 cells [MCF7], WSTF stably expressing MCF7 cells [WSTF stable] and MCF7 cells transfected with WSTF-RNAi [i-WSTF]. Right panel: BrdU incorporation during S phase of the MCF7 cells transfected with RNAi from the indicated proteins during double thymidine treatment. After the final release (time 0), cells were collected every 2 h, for up to 8 h. The average values of triplicate analyses are shown.


(A)
GAL4-VDR-DEF
GAL4-ERα-DEF
transcriptional activity(fold)
WSTF
WSTF- RNAi
control-RNAi
Brg1
hBrm
TRAP220
TIF2
hSNF2h
Ligand
D3
E2
(B)
24(OH)ase
1α(OH)ase
distal
positive VDRE
negative VDRE
input
αVDR
αfWSTF
αBrg1
αhBrm
αTRAP220
αTIF2
αAcH4
αhSNF2h
αN-CoR
αHDAC2
D3
WSTF-RNAi
(C)
24(OH)ase promoter
transcriptional activity(fold)
WSTF
WSTF-RNAi
Brg1
hBrm
TRAP220
TIF2
D3
(D)
1α(OH)ase promoter
transcriptional activity(fold)
WSTF
WSTF-RNAi
Brg1
hBrm
N-CoR
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WSTF
VDR
GAPDH

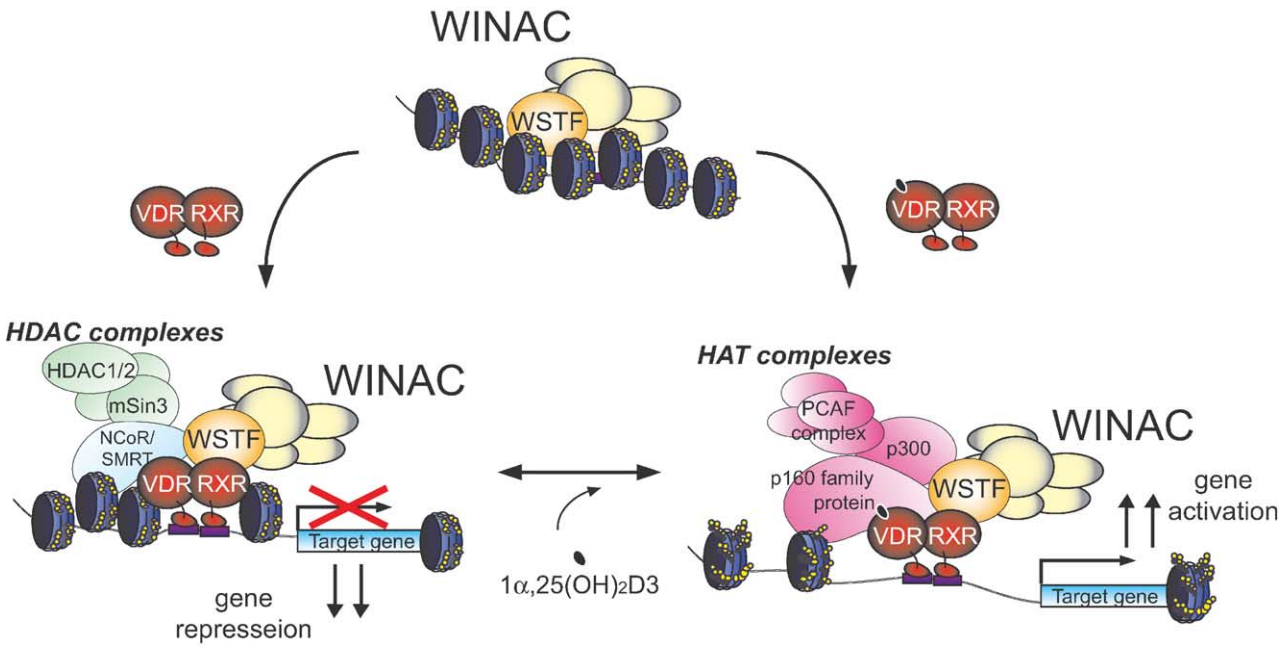

Fig. 3. Schematic illustration of WINA function in VDR-mediated gene regulations with 10-regulatory complexes.

ligand-induced TRAP220 and TIF2 occupancy was cyclic (data not shown) as expected from previous reports [20].

As the VDR/RXR heterodimer also represses transcription in a ligand-dependent manner through negative VDRE (nVDRE), the action of WSTF in the ligand-induced transrepression was examined in a naturally occurring nVDRE in human 25-hydroxyvitamin D3 1α-hydroxylase [1α(OH)ase [22]. ChIP analysis uncovered that VDR and WINAC appear to land on the nVDRE in a ligand-independent manner, while ligand-induced, but cyclic (data not shown) recruitments of N-CoR and HDAC2 were observed. Ligand-dependent repression was exaggerated by WSTF overexpression, but attenuated again by WSTF-RNAi expression (Fig. 2B). Thus, it is likely that WINAC association with VDR facilitates targeting of a putative co-repressor complex to the nVDRE. Thus, these findings point out that WINAC rearranges the nucleosome array around the positive and negative VDREs, thereby facilitating the co-regulatory complexes accessible to VDR for further transcription control.

5. Promoter targeting of VDR by WINAC, with cooperation of WINAC with the co-regulator complexes

From our ChIP analysis, VDR appears to be selectively targeted through WINAC to the promoters without ligand-induced activation of VDR function or following recruitment of co-regulator complexes. This ligand-independent association with the target promoter in VDR is unexpectedly distinct from the ligand-induced promoter targeting of many steroid receptors [20,21]. It is possible to speculate that the other non-steroidal receptors like RAR, RXR and TR may associate with their target promoters without ligand binding, and unidentified chromatin remodeling complexes may assist the promoter targeting.

As HAT and HDAC complexes appears not to associate with unliganded VDR, WINAC targeting to the VDR target promoters appears not to require specific histone tail modifications by the co-regulators (Fig. 3). Thus, it is likely that WINAC associating on promoters escort VDR for its

Fig. 2. Ligand-dependent promoter targeting of co-regulators through WINAC-VDR association. (A) VDR-specific facilitation of co-activator accessibility by WINAC. MCF7 cells were transfected with the expression vectors of a luciferase reporter plasmid containing the GAL4 upstream activation sequence (UAS) [17mer(x2)] driven by the b-globin promoter (0.5 μg). PML-CMV (2 ng), GAL4-DBD-VDR-DEF (0.2 μg), GAL4-DBD-Era-DEF (0.2 μg), pDNA3-FLAG-WSTF (+: 0.1 μg; ++: 0.3 μg), pSV-Brg1 (0.2 μg), pSV-hBrm (0.2 μg), pcDNA3-TRAP220 (0.3 μg), pcDNA3-TIF2 (0.3 μg), siRNA (+: 0.1 μg; ++: 0.2 μg) of WSTF-RNAi, or control RNAi or their combinations were transfected as indicated in the panels in the absence or presence of ligand (10^{-9} M). Bars in each graph show the fold change in luciferase activity relative to the activity of the receptor transactivation in the presence of ligand. (B) ChIP analysis on the 24(OH)ase promoter and 1α(OH)ase promoter of WSTF stable transformants. Soluble chromatin was prepared from WSTF stable transformants treated with D3 (10^{-9} M) for 45 min and immunoprecipitated with indicated antibodies. (C and D) The co-regulator-like actions of WSTF on the naturally occurring positive and negative Vitamin D response elements. MCF7 cells were transfected with the expression vectors of either the luciferase reporter plasmid containing a human 24(OH)ase promoter harboring a canonical positive VDRE or a human 1α(OH)ase promoter containing a negative VDRE and the factors shown in (A) or together with pcDNA3-*N*-CoR (0.3 μg), pcDNA3-HDAC2 (0.3 μg). (E) WSTF-mediated regulations of endogenous genes by VDR. RT-PCR analysis of MCF7 cells was performed 12 h after the induction by D3 (10^{-9} M) [5].

recognition and specific binding to VDREs, through nucleosomal mobilization by WINAC, presumably co-operating with the other chromatin complexes [19]. Alternatively, once VDR happens to bind VDREs during non-specific chromatin remodeling, WINAC might be acquired to VDR upon the promoters to engage in local nucleosome reorganization [14].

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