Nuclear Receptors: a rendezvous for chromatin remodeling factors

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Summary

Nuclear receptors (NRs) are a large family of ligand-induced transcription factors that includes the vitamin D receptor. The recent discovery of WINAC, a novel ATP-dependent chromatin remodeling complex, has shed new light about the molecular mechanisms by which the vitamin D receptor control gene expression with unexpected clinical implications.

In eukaryotic organisms, the packaging of DNA in the form of nucleosomes to generate chromatin is an effective way of storing DNA inside the cell nucleus but it poses formidable problems for the transcription of genes and the replication of the genome. The formation of a tightly packed chromatin fiber leads to a repressive environment that has to be overcome to allow these essential functions to take place. This is achieved by a rather ill-defined process termed chromatin remodeling that regulates the accessibility of gene promoters to the transcription and replication machinery. The number of chromatin remodeling enzymes is extensive but they can be divided into two distinct classes, both of which contribute to the generation of a dynamic chromatin structure. First, there are ATP-dependent complexes that are involved in the location and association of nucleosomes with DNA and second there are enzymes that catalyze post-translational modifications in histones. Such modifications are proposed to constitute a 'histone code' that represents an epigenetic marking mechanism for controlling gene transcription and other chromatin-regulated processes (Jenuwein and Allis, 2001).

The progress made in identifying chromatin remodeling complexes over the past decade has been astonishing. Additional complexes continue to be discovered, including a novel ATP-dependent remodeling complex, termed WINAC, described recently by Kato and coworkers in Cell to play a role in vitamin D action (Kitagawa et al., 2003). Despite many advances in the characterization of remodeling complexes fundamental questions remain to be answered. For example, the rules that govern which remodeling complexes are required to regulate the transcription of specific sets of genes remain to be established, details of their mode of recruitment are just emerging but the kinetics of the process are controversial and little is known about their precise function in regulating transcription from endogenous genes. Studies in yeast are clearly the most tractable and they are providing a basic understanding of the fundamental principles of transcriptional regulation. Studies in higher eukaryotic organisms are more complex but a number of hormonally and

developmentally regulated genes are amenable to a detailed analysis using cell-free reconstitution assays, mouse models and, very occasionally, clinical observations. The ability of hormones to trigger the activation or repression of NRs provides an ideal opportunity for investigating the transcription of specific target genes both *in vitro* and *in vivo*.

NRs comprise a family of ligand-dependent transcription factors that includes the vitamin D receptor. Over the past ten years many cofactors implicated in their transcriptional regulation have been identified (McKenna and O'Malley, 2002) (Table 1). Amongst the best characterized are the p160 coactivators which seem to serve as platforms for the recruitment of histone modifying enzymes, including CBP/p300 and methyltransferases that acetylate or methylate histones, respectively. The p160 proteins have been found to interact directly with an activation surface, called AF2, located in the ligand binding domain of NRs. Similarly, a number of corepressor proteins such as NCoR, SMRT, RIP140 and LCoR may also be recruited to this surface of the receptor, dependent on the ligand bound. They too seem to function as platforms, but serve to recruit enzymes such as histone deacetylases or redox sensing cofactors including C-terminal binding protein (CtBP). The nature of the cofactors recruited to a target promoter is envisioned to determine its transcriptional activity.

A role for ATP-dependent chromatin remodeling complexes in transcriptional regulation by steroid receptors was first proposed by Yamamoto (Yoshinaga et al. 1992) and substantiated by Yaniv (Murchard and Yaniv, 1993). The precise number of ATP-dependent chromatin remodeling complexes is unknown but there are at least four families each containing a distinct core ATPase subunit, SWI/SNF, ISWI, CHD and INO80 (Becker and Hörz, 2002). To this list must be added the WINAC complex, which shares subunits with two distinct family members, SWI/SNF and ISWI. They function as multiprotein complexes comprising up to 10-12 subunits with roles not only in gene transcription but

also in DNA replication, DNA repair and recombination. The SWI/SNF and ISWI families are the two best characterized ATP-dependent chromatin remodeling complexes. Both classes have been shown to induce nucleosome sliding along DNA, although their biological functions and biochemical activities are different. In addition, SWI/SNF can cause conformational changes in DNA on the surface of the nucleosome and it can remodel topologically constrained chromatin templates. An interaction between the glucocorticoid receptor and the SWI/SNF remodeling complex was demonstrated by Archer (Fryer and Archer, 1998) and this was subsequently found to be mediated by BAF250, a subunit, present only in a subset of SWI/SNF complexes (Nie et al., 2000). The estrogen receptor has been found to interact with another component found in all SWI/SNF remodeling complexes, namely BAF57, (Belandia et al., 2002) and this appears to be essential for the ability of the p160 proteins to potentiate transcription from estrogen-responsive reporter genes.

Several lines of evidence indicate a close functional link between SWI/SNF complexes and histone acetyltransferases. While SWI/SNF can be recruited directly to chromatin by transcription factors, the acetylation of histone tails has been found to stabilize its binding to nucleosomes. This strong interaction, which is mediated by a bromodomain, can then be maintained even in the absence of the transcription factor that initially recruited the coactivator complex (Jenuwein and Allis 2001, Hassan et al., 2002). The functions of a number of remodeling complexes in transcriptional regulation by NRs has been examined in chromatin-dependent transcription assays (Dilworth et al., 2000, Lemon et al., 2001, Huang et al., 2003). Interestingly, Chambon and coworkers found that the ability of retinoid receptors to stimulate transcription depended on the sequential action of a number of remodeling complexes, starting with ISWI, followed by histone aceltyltransferases, specifically a p160-CBP/p300 complex, and finally SWI/SNF. This observation therefore supports the view that histone acetylation is required for remodeling

chromatin by the SWI/SNF complex. A functional link between the two families of coactivators is also evident from the recent analysis of their function in promoting the transcriptional activity of both the androgen and the thyroid hormone receptors (Huang et al., 2003). Wong and coworkers confirmed that the p160 proteins are necessary for the recruitment of CBP/p300 but then demonstrate that these alone do not lead to chromatin remodeling but rather to the recruitment of SWI/SNF which is capable of inducing chromatin remodeling. Thus it appears that there are multiple protein-protein interactions between coactivators and NRs and between coactivators themselves. A distinct ATP-dependent remodeling complex has been found to repress gene transcription, namely NURD, a member of the chromodomain family of complexes. NURD contains at least two HDACs and is recruited to the estrogen receptor by means of MTA1, an integral component of the complex, whereupon it repressed ligand-dependent transcription by the estrogen receptor (Mazumdar et al., 2000).

In a recent issue of Cell, Kitagawa et al. describe a novel multi-functional ATP-dependent chromatin remodeling complex called WINAC that interacts directly with the vitamin D receptor. WINAC represents a new member of the SWI/SNF subfamily with unique characteristics. It contains BRG1 or hBRM as ATPase subunits like all SWI/SNF complexes, but it also has subunits associated with DNA replication (TopoIIβ, and CAF-1p150) and transcript elongation through nucleosomes (FACTp140) not found before in SWI/SNF complexes. In addition, WINAC also contains the Williams syndrome transcription factor (WSTF). The *WSTF* gene is deleted in patients with Williams syndrome, a complex neurodevelopmental disorder caused by heterozygous deletions of 1.6 Mb at chromosome 7q11.23. This syndrome is characterized by congenital vascular and heart disease, dysmorphic facies, mental retardation, growth retardation, infantile abnormal vitamin D metabolism and hypercalcemia (Morris and Mervis, 2000). WSTF, like the closely related ACF1, was recently reported to be associated with hISWI (otherwise known

as hSNFH and hSNFL) forming a distinct ATP-dependent chromatin remodeling complex termed WICH (Bozhenok et al., 2002). WICH and other ISWI complexes mobilize nucleosomes and create regular nucleosomal arrays from irregular chromatin. WINAC promotes both the assembly and disruption of nucleosome arrays in an ATP-dependent manner and in addition, like the CAF-1 histone chaperone complex, it can also reconstitute chromatin upon newly replicated DNA.

WSTF appears to function as a platform protein for the assembly of components in WINAC and it is also capable of interacting directly with the vitamin D receptor. The interaction between vitamin D receptor and WINAC occurs in a ligand-independent manner, in contrast to that between glucocorticoid receptor-BAF250 and estrogen receptor-BAF57, which are promoted by ligand binding. Chromatin immunoprecipitation experiments confirmed that WINAC and vitamin D receptor are targeted to vitamin D responsive promoters in the absence of ligand, interestingly to both positively and negatively regulated genes. Manipulation of the levels of WSTF expression established that this subunit is essential for vitamin D receptor both to stimulate transcription from promoters regulated by positive response elements and repress transcription from promoters containing negative response elements. The molecular basis for this dual function is unknown but it is conceivable that the sequence of the individual positive and negative vitamin D receptor response elements might act as an allosteric effector of receptor conformation (Koszewski et al., 1999), facilitating the binding of distinct enzymatic activities associated with coactivators or corepressors. WINAC, like other SWI/SNF and ISWI complexes, seems to be able to reorganize the chromatin fiber either way, opening the structure to allow transcription or compacting it to repress gene expression.

Vitamin D is essential for mineral homeostasis, skeletal integrity and control of cell growth and differentiation in many tissues (Sutton and MacDonald, 2003). Ascribing Williams syndrome abnormalities to defects in the ability of vitamin D receptor to regulate

transcription is difficult and complicated by the fact that the syndrome is caused by heterozygous deletions of 1.6 Mb, which includes not only WSTF but also at least 15, other genes. In fact, given a defect in vitamin D signaling, certain features such as hypercalcemia are paradoxical because one of the functions of vitamin D is to promote calcium uptake. However this might be explained by the observation that WSTF is required for the expression of enzymes involved in both the synthesis and the catabolism of vitamin D. An abnormal vitamin D receptor function could be also at the root of some of the cardiovascular problems. Haploinsuffienciency for *elastin* gene seems to be the main cause of the arterial pathology, however its expression is dependent on vitamin D intake (Li et al., 1998 and references therein). Therefore, impaired vitamin D receptor function could contribute to the generation of a more severe cardiovascular disease. At the present time it is not be possible to draw definitive conclusions about the contribution of vitamin D receptor signaling to the abnormalities observed in Williams syndrome patients, but a comparative analysis of mice with tissue specific deletions of vitamin D receptor and WSTF alleles should help to elucidate their biological roles and may provide insights into certain aspects of Williams syndrome.

Our understanding of the function of NRs has been dramatically advanced over the past decade by using biochemical approaches to investigate structure-function relationships and by studying genetically manipulated mice, particularly gene deletions. Tremendous progress has also been made to elucidate the function of chromatin remodeling complexes in transcriptional regulation, particularly using reconstituted systems, and the biological roles of these complexes are beginning to emerge from studies of mice devoid of specific genes. However, functional redundancy is a confounding factor and interpreting the phenotypes is often not straightforward especially for those coactivators with global effects. Nevertheless, by focusing on key target genes for NRs, further advances will undoubtedly

be made. The challenge therefore will be to use these clues to determine the origin of many human diseases and help to develop therapeutic strategies.

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Figure legend

Figure 1. Proposed models for the contribution of WSTF haploinsufficieny to Williams syndrome phenotype. WSTF, when incorporated into WINAC, is required for the transcriptional regulation by vitamin D receptors (VDR) both in negatively (A) and positively (B) regulated target genes. WINAC mediates the recruitment of unliganded vitamin D receptor to vitamin D response elements (VDREs) while subsequent binding of coregulators requires ligand binding. It is not known yet whether WINAC and other coregulators interact simultaneously or sequentially with the vitamin D receptor. Aberrant expression of WSTF can affect the recruitment of WINAC and other chromatin modifying enzymes to vitamin D responsive genes, altering both basal and hormone-dependent gene expression. As a consequence of defective vitamin D signaling, calcium and vitamin D metabolism in addition to many other metabolic processes will be disturbed. Insufficient WSTF in the cell might also impair DNA replication (C), which requires WINAC and the WICH complex (hISWI-WSTF), affecting cell cycle regulation and/or chromosome stability. The effects on transcriptional control and DNA replication are not mutually exclusive and both could contribute to the complex phenotype observed in Williams syndrome patients. For more details see text and Table 1.

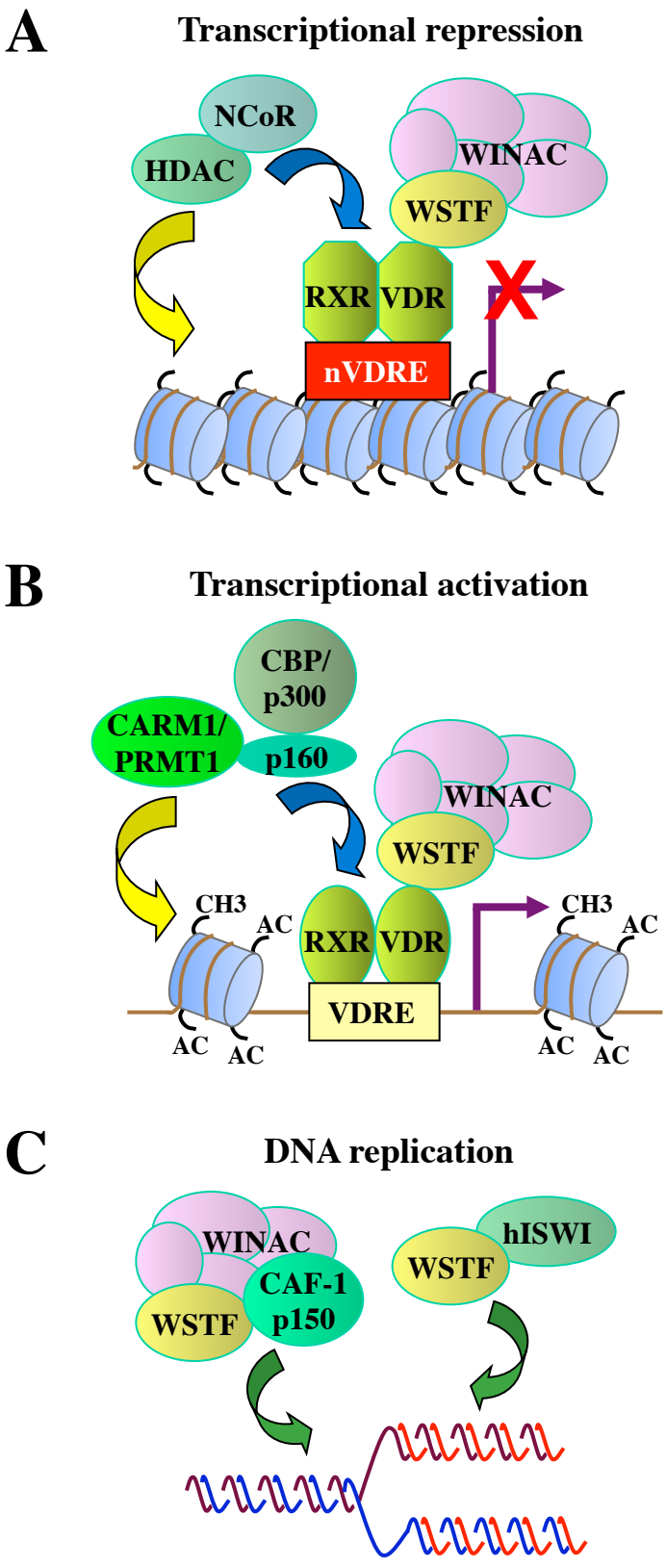
A
Transcriptional repression
NCoR
HDAC
WINAC
WSTF
RXR
VDR
nVDRE
B
Transcriptional activation
CBP/
p300
CARM1/
PRMT1
p160
WINAC
WSTF
CH3
AC
RXR
VDR
VDRE
AC
AC
CH3
AC
AC
AC
C
DNA replication
WINAC
CAF-1
p150
WSTF
hISWI
WSTF

Table 1. Protein interactions recruiting chromatin modifying activities to nuclear receptors

Name	Type of activity	Direct interaction with NRs and subunits involved	Bridging factors	Role in activation	Role in repression
p160 CoA	Lysine acetyltransferases	+	-	+	-
CBP/p300	Lysine acetyltransferases	+	p160	+	-
PCAF	Lysine acetyltransferases	+	p160	+	-
TRRAP/GCN5	Lysine acetyltransferases	TRRAP-ER	-	+	-
INHAT	Inhibitor of acetyltransferases	?	?	-	+
PRMT1	Arginine methyltransferase	-	p160	+	-
CARM1	Arginine methyltransferase	-	p160	+	-
HDACs	Lysine deacetylases	-	NCoR, SMRT RIP140, LCoR ALIEN, PSF	-	+
SWI/SNF class	ATP-dependent chromatin remodeling	BAF57-ER BAF250-GR	p160 ARA160	+	+
ISWI class	ATP-dependent chromatin remodeling	?	?	+	+
WINAC	ATP-dependent chromatin remodeling	WSTF-VDR	-	+	+
NURD	ATP-dependent chromatin remodeling + lysine deacetylase	MTA1-ER	-	-	+