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Biochem Cell Biol. Author manuscript; available in PMC 2012 January 4.

Published in final edited form as:

Biochem Cell Biol. 2011 February ; 89(1): 12–23. doi:10.1139/O10-114.

WSTF does it all: a multifunctional protein in transcription, repair and replication

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Abstract

Williams Syndrome Transcription Factor (WSTF) has emerged as an incredibly versatile nuclear protein. WSTF and the ATP-dependent chromatin remodeling complexes in which it exists, WINAC, WICH and B-WICH have been studied in a variety of organisms. This research has revealed roles for WSTF in a number of diverse molecular events. WSTF function includes chromatin assembly, Pol I and III gene regulation, vitamin D metabolism and DNA repair. In addition to functioning as a subunit of several ATP-dependent chromatin remodeling complexes, WSTF binds specifically to acetylated histones and is itself a histone kinase, as well as a target of phosphorylation. This review will describe the three known WSTF-containing complexes and discuss their various roles as well as mechanisms of regulating WSTF activity.

Keywords

WSTF; WICH; WINAC; B-WICH; ATP-dependent Chromatin Remodeling Complex; Vitamin D; DNA Repair; H2A.X; Tyrosine 142

ATP-dependent Chromatin Remodeling Complexes

The eukaryotic genome exists in a highly compact form inside the nucleus of each cell. The nucleosome is the foundation of this condensed DNA structure within the cell. The nucleosome consists of 147 base pairs of DNA wrapped about 1.7 times around a histone octamer. Each histone octamer is comprised of two copies of histone H2A, H2B, H3 and H4 (Luger et al. 1997). The compaction state of DNA is dynamic, and regions of chromatin can exhibit vastly different folding levels during the cell cycle and during transcription and repair. Heterochromatin displays far less transcriptional activity than euchromatin and formation is closely associated with long term gene silencing (Rizzi et al. 2004). Heterochromatin formation creates a physical barrier between DNA and transcription machinery that acts to suppress gene expression (Grewal and Jia 2007). Nucleosomes within euchromatic regions also play an important role in gene regulation (Wyrick et al. 1999). Researchers performing high-density tiling arrays discovered that promoters typically reside in nucleosome-free regions, providing evidence that nucleosomes are not assembled onto DNA at random, or even in the most energy favorable conformation (Yuan et al. 2005). The current understanding of chromatin formation is that nuclesomes not only exist to organize and package DNA, but are also specifically positioned along the genome to help regulate gene expression (reviewed in Venters and Pugh 2009).

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For molecular machinery involved in transcription, replication and repair to gain access to DNA, nucleosomes must first be displaced to expose specific segments of DNA. Nucleosomal movement and nucleosomal eviction facilitate this exposure, allowing for molecular machinery to directly associate with the DNA (Vignali et al. 2000). The molecular motors responsible for nucleosome positioning and displacement are known as ATP-dependent chromatin remodelers. These nuclear complexes utilize the energy of ATP hydrolysis for assembly, repositioning, or eviction of nucleosomes, therefore exposing (or covering) specific sections of DNA as necessary for transcription, replication, or repair (reviewed in Venters and Pugh 2009). All ATP-dependent chromatin enzymes are a part of the helicase super family 2 (SF2) because of structural similarities between the ATPase and the helicase domains (Bouazoune and Brehm 2006), though these complexes do not exhibit conventional helicase activity. Chromatin remodeling complexes are classified into subfamilies based on the specific ATPase the complex utilizes. SWI/SNF and ISWI are two of the most extensively studied subfamilies of ATP-dependent remodelers. SWI/SNF and ISWI complexes are thought to achieve nucleosome displacement in different ways. SWI/SNF remodelers expose gene segments by creating DNA loops without transferring the nucleosomes to another region (Fan, *et al*. 2003). ISWI complexes widen inter-nucleosomal regions by sliding nucleosomes, creating longer segments of linker DNA (reviewed in Gangaraju and Bartholomew 2007). SWI/SNF complexes have both activation and repressive functions and tend to be larger complexes, containing up to 15 subunits. ISWI complexes generally contain fewer subunits, often 2–4 and are commonly involved in remodeling chromatin to repress gene activity (reviewed in Dirscherl and Krebs 2004; Gangaraju and Bartholomew 2007; Lall 2007).

Histone modifications

Chromatin remodelers typically lack the innate ability to recognize specific DNA sequences and therefore are directed to their sites of action by sequence-specific DNA binding proteins and by histone marks left by histone modifying enzyme complexes (Heinzel et al. 1997; Yanagisawa et al. 2002). These enzyme complexes possess the ability to add or remove post-translational modifications primarily to the flexible terminal tails of histone proteins, though modifications within the histone core domains also occur. The vast array of modifications that have been identified include phosphorylation, acetylation, methylation, ubiquitination, sumoylation, ADP-ribosylation, deimination and proline isomerization (the last being a unique case of a non-covalent modification; (reviewed in Kouzarides 2007)).

These modifications can function by altering the chromatin structure itself, or by the recruitment of other nuclear proteins and complexes to specific sites of the genome. A classic example of a histone modification resulting in the recruitment of nuclear machinery is γ-H2A.X. H2A.X is a variant of histone H2A shown to play a crucial role in DNA damage response (Xiao et al. 2009). γ-H2A.X refers to the phosphorylated state of a specific serine residue, serine 139 in most multicellular organisms (S129 in yeast). This specific modification of H2A.X is essential for damage focus formation at sites of DNA double strand breaks (Rogakou et al. 1998; Rogakou et al. 1999; Paull et al. 2000). γ-H2A.X is recognized by MDC1 (**M**ediator of **D**NA damage **c**heckpoint protein **1**), a mediator protein that recruits a number of other proteins that help coordinate the assembly of DNA damage response (DDR) proteins (Stucki 2009). A number of remodeling complexes also appear to be recruited to sites of DNA damage. INO80 is remodeling complex that binds γ-H2A.X in yeast and is believed to be responsible for the nucleosomal displacement at sites of double stand breaks (Morrison et al. 2004; Wong et al. 2006). The interaction between modified histones and chromatin remodelers allows for the manipulation of nucleosomes in a targeted region. This interplay allows remodelers to establish widespread chromatin conformations

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(euchromatin and heterochromatin) as well as coordinate the regulation of specific gene availability (reviewed in Kouzarides 2007).

This review will focus on WSTF (**W**illiams **S**yndrome **T**ranscription **F**actor; Figure 1) and the functions of the three chromatin remodeling complexes in which it exists: WINAC, B-WICH and WICH (shown schematically in Figure 2). WSTF is a versatile nuclear protein that plays roles in both chromatin remodeling and covalent histone modification.

WSTF and Williams Syndrome (WS)

WSTF is a known subunit of three ATP-dependent chromatin remodelers: WINAC (**WS**TF **i**ncluding the **n**ucleosome **a**ssembly **c**omplex), WICH (**W**STF-**I**SWI **ch**romatin remodeling complex) and B-WICH. The gene that codes for WSTF (*WBSCR*) was first identified by researchers studying a 1.5 megabase heterozygous deletion of roughly twenty genes on chromosome seven, seen in all patients with Williams Syndrome (Ewart et al. 1994). WS is a rare autosomal dominant hereditary disorder that occurs in about 1 of every 20,000 live births (Lu et al. 1998). Patients suffering from Williams Syndrome may present a number of systemic defects including characteristic personality and facial appearance, growth deficiency, mental retardation, aortic stenosis, heart disease and infantile hypercalcaemia (Bellugi et al. 1990; Morris et al. 1988; Pober 2010). Although over 20 other genes reside within the chromosome 7q11.23 deletion, studies in *Xenopus,* mouse and tissue culture have lead researchers to believe that WSTF heterozygosity plays an important role in a number of the systemic defects seen in WS patients (Bozhenok et al. 2002; Cus et al. 2006; Yoshimura et al. 2009).

WSTF Expression and Role in Development

The first examination of *WSTF* expression was done by northern blot analysis of human tissue. This revealed a 7.5-kb transcript expressed in all adult tissues (heart, brain, placental, skeletal, muscle and ovary) and four fetal tissues (brain, lung, kidney and liver) examined in non-WS individuals (Lu et al. 1998). Due to the diversity of defects seen in patients with WS, researchers have investigated *WSTF* expression during several stages of vertebrate development. *In situ* hybridization assays of developing *Xenopus laevis* embryos reveal that *WSTF* mRNA is detected in *Xenopus* oocytes and is ubiquitous until stage 16 (about 24 hours post-fertilization). *WSTF* expression first begins to localize to the closing neural tube. As neural tissue continues to differentiate into early tadpole stage, *WSTF* mRNA localizes to specific neural structures and cells including the neural tube, migrating head neural crest cells, anterior brain and optic cup ((Cus et al. 2006) and our unpublished data). *WSTF* expression is strongly detected in the optic cup of *Xenopus* embryos, but absent within the developing lens epithelium (Cus et al. 2006). In *Xenopus* embryos at tadpole stage (stages 32–39), *WSTF* expression within the optic cup becomes limited to the ciliary marginal zone, a regenerative structure absent in higher order vertebrates (Kubota et al. 2002). Also at this stage *WSTF* mRNA is strongly detected in the mid and hindbrain as well as the tectum and isthmus, structures shown to have organizing activity during brain development (Cus et al. 2006; Nakamura et al. 2005). Strong WSTF expression is detected within the migrating head neural crest cells and also within the branchial arches. The branchial or pharyngeal arches are a primordium for a number of organs and facial structures, such as the thyroid, aorta, the maxillary and mandibular bones, ligaments, and nerves as well as auditory structures like the auditory tube and the epithelium around the ear. *WSTF* mRNA is also faintly detected in the tailbud of these tadpole embryos. *WSTF* transcript detection persists within these defined domains until late tadpole stage (stage 39), where expression begins to fade until *WSTF* transcripts are no longer detectable by *in situ* hybridization (stage 42) (Cus et al. 2006). *WSTF* expression has been shown to have significant overlap with members of both WINAC

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and WICH complexes in *Xenopus laevis* (Dirscherl et al. 2005; Linder et al. 2004). However, researchers have also shown a distinct difference between the expression patterns of *WSTF* and WICH complex member *ISWI* in certain developmental stages of mice. This may reveal important differences in the tissues where the WICH and WINAC complexes assemble and highlight the potential for unique roles for these two WSTF-containing remodeling complexes during development (Kitagawa et al. 2003). Researchers have also noticed strong overlap of *WSTF* mRNA with Wnt pathway ligand *Wnt-4* and transmembrane Wnt receptor frizzled-3 (*Frz-3*) during *Xenopus* development (Cus et al. 2006; Lyuksyutova et al. 2003). Both *Wnt-4* and *Frz-3* are expressed in domains similar to that of *WSTF* and both are neural-specific genes shown to be involved in eye development (Maurus et al. 2005; Rasmussen et al. 2001).

WSTF is an essential gene in mice as WSTF homozygous knockouts (*WSTF*−/−) develop with smaller body size and die just days after birth. *WSTF*−/− embryos show significant heart defects and 10% of *WSTF*+/− neonatal mice also display specific heart defects such as altered structure, expansion of the septal defects and hypertrophy of ventricles similar to those seen in WS patients (Yoshimura et al. 2009).

Whole mount *in situ* hybridizations reveal that *WSTF* mRNA is detectable in the headfolds, presumptive hindbrain and in the caudal tail bud of mice at 8.25 days postcoitum (dpc). At 9.5 dpc, *WSTF* mRNA is detected in the somites as well as the budding forelimb. *WSTF* expression is also present in the mesenchymal tissue of brachial arches 1 and 2, which contribute greatly to maxillary and mandibular structures of the face. WSTF expression can also be detected in the frontonasal process at 9.5 dpc as well as in the mesenchyme of medial and lateral prominences from 10.5 – 11.5 dpc (Ashe et al. 2008). These studies reveal robust *WSTF* expression in a number of structures critical for facial development throughout early embryogenesis in mice.

A WSTF mutant was obtained in a screen in mice for *Modifiers of murine metastable epialleles* (*Mommes*) (Ashe et al. 2008). The *MommeD10* mutation was identified as a single amino acid change (L733R) within the WSTF protein. Even though *WSTF* transcript levels are normal in *MommeD10* mutant mice, this mutation appears to disrupt protein stability as WSTF protein levels are severely diminished. Unlike *WSTF−/−* null animals, *MommeD10* mice are able to survive and at 4 weeks exhibit an altered skull shape that includes protruding foreheads, shorter snouts, flattened nasal bone, and upward curvature of the nasal tip (Ashe et al. 2008). These craniofacial features are stirkingly similar to those of WS patients. This similarity, as well as the detection of *WSTF* expression in structures known to be crucial for facial morphology in both mice and *Xenopus laevis* strongly suggest that reduced expression WSTF may be responsible for the characteristic “elfin” features seen in WS individuals.

WSTF Protein

The WSTF protein falls into the BAZ/WAL family, a group of proteins that include six specific motifs found in sequential order (Jones et al. 2000; Poot et al. 2000): an LH motif, a proposed transactivation domain (Quong et al. 1993), BAZ 1, which mediates WSTF association with ISWI, BAZ 2 motifs, a WAKZ motif (Ito et al. 1999; Jones et al. 2000; Quong et al. 1993), a PHD finger, and a bromodomain (Jones et al. 2000). WSTF also contains an N-terminal WAC domain that does not exist in all BAZ proteins (Figure 1).

Studies with HeLa cells reveal that WSTF shows close sequence homology with a number of known subunits of other ISWI complexes, many of which fall in the BAZ/WAL family (Bochar et al. 2000). These non-catalytic subunits are believed to facilitate specific interaction and functions with chromatin (He et al. 2008). For example, ACF1 enhances

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nucleosome sliding and chromatin assembly by ISWI in *Xenopus* and ACF1-deficient cells show a reduction in chromatin bound ISWI in *Drosophila* (Guschin et al. 2000; Ito et al. 1999; Yoshimura et al. 2009).

Several protein domains in WSTF are implicated in binding and/or modification of chromatin. These include the PHD (**P**lant **h**omeo**d**omain) finger, the bromodomain (Bochar et al. 2000) and a histone kinase activity composed of the WAC domain and additional N-terminal sequences (Xiao et al. 2009). Proteins that contain PHD fingers, which are common methyl-lysine binding motifs, tend to have a role in transcription and are therefore almost exclusively located in the nucleus. hWSTF contains a single PHD finger that is about 50 amino acids in length and is defined by a Cys4-HisCys3 zinc binding motif (Aasland et al. 1995; Pascual et al. 2000). The hWSTF PHD finger is a β-β-α core metal-dependent folding motif that specifically binds two zinc ions (Pascual et al. 2000). The PHD finger of BPTF (**b**romodomain and **P**HD domain **t**ranscription **f**actor), a subunit of the ISWI containing chromatin remodeling complex NURF (**nu**cleosome **r**emodeling **f**actor), preferentially binds trimethylated lysine 4 on histone 3 (H3K4me3), a histone modification that correlates with the starts sites of active genes (Li et al. 2006). The PHD finger of the Mi-2 subunit of NuRD (**nu**cleosome **r**emodeling **d**eacetylase), another ISWI containing complex, reveals an interaction with histone deacetylase and a connection with gene repression (Zhang et al. 1998). The specific binding target of the WSTF PHD finger remains unknown but is likely to play a role in targeting WSTF complexes.

Adjacent to the PHD finger of WSTF is a bromodomain. The bromodomain was first described as an acetylated lysine-binding domain in the histone acetyltransferase P/CAF (p300/CBP-associated factor) (Dhalluin et al. 1999). Acetylated lysine is an essential modification of histone tails that is important in gene activation (Winston and Allis 1999). The WSTF bromodomain binds acetylated lysine 14 on histone 3 (AcH3K14) and exerts a transrepressive function on gene activation (Kato et al. 2007). The results of this transrepressive activity will be described in further detail later in this review.

BPTF, like ACF1, WSTF and WCRF180 are all members of different ISWI complexes, each containing both a PHD finger and a bromodomain, suggesting that they may have similar functional roles (Bochar et al. 2000; Ito et al. 1999; Li et al. 2006; Strohner et al. 2001). The ability of these domains to recognize and bind specific modifications on histone tails provides these proteins the capacity to distinguish between nucleosomes and decipher the epigenetic marks of the histone code (Ragvin et al. 2004). It is likely that these domains facilitate these functions of WSTF and contribute greatly to its role within a complex.

In addition to the motifs within WSTF that may facilitate its binding to specific histone marks, WSTF can also covalently modify histone targets. A recent discovery revealed an intrinsic tyrosine kinase activity of the WAC domain (plus an additional N-terminal region) of WSTF in cultured MEF (**m**ouse **e**mbryonic **f**ibroblast) cells (Xiao et al. 2009). The WAC domain is not a motif that exists in all BAZ/WAL proteins (Bozhenok et al. 2002). This kinase domain has no sequence homology to any other known kinases, and has a very interesting target. WSTF phosphorylates tyrosine 142 of histone H2A.X and this modification plays a crucial role in the DNA damage response. This is described further below.

Post-translational modifications of WSTF

In addition to its ability to both recognize and catalyze histone modifications, WSTF itself is post-translationally modified. The WSTF protein has recently been shown to be a target of MAP kinases both *in vitro* and *in vivo* (Oya et al. 2009). MAPK phosphorylates WSTF protein within the WAC domain at serine 158 (S158). While WSTF exists in both the

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phosphorylated and unphosphorylated form *in vivo*, researchers have shown that an unphosphorylatable WSTF mutant (WSTF S158A) displays a significantly reduced affinity for other components of the WINAC chromatin remodeling complex. This mutation also results in reduced WINAC transactivation and repression activity in the breast cancer cell line MCF7 (Oya et al. 2009). Interestingly, WSTF S158A does not disrupt the association with ISWI and appears not to alter survival of cultured mouse embryonic fibroblast (MEF) cells upon treatment with MMS. This finding reveals a post-translational modification of WSTF that is important for WINAC assembly and function, but not for WICH (Figure 3). It is possible that a MAPK-dependent switch mechanism exists between WICH and WINAC assembly through the post-translational phosphorylation of the WSTF WAC domain, and that WINAC-dependent chromatin remodeling is at least partly under MAPK control (Oya et al. 2009). It is intriguing that the WAC domain is essential for WSTF's H2A.X kinase activity; it will be interesting to see whether S158 phosphorylation impacts the kinase activity of WSTF.

WINAC

WSTF is one of 13 subunits that make up the ATP-dependent chromatin remodeling complex WINAC (**W**STF **i**ncluding **n**ucleosome **a**ssembly **c**omplex) (Figure 2A) (Kitagawa et al. 2003). This complex contains SWI/SNF ATPases Brg1 and Brm, a number of BAF (**B**rg1- **a**ssociated **f**actor) subunits, as well as TopoIIβ, FACT p140, and CAF-1 p150 (Kitagawa et al. 2003). Topoisomerase II (Topo II) is a nuclear enzyme largely responsible for the remove helical tension of DNA (Salceda et al. 2006). FACT p140 is a subunit of the FACT (**Fa**cilitates **c**hromatin **t**ranscription) complex known to be required for Pol II transcript elongation (Belotserkovskaya et al. 2003). CAF-1p150 is a subunit of the multiprotein complex CAF-1, known for its role in chromatin assembly during DNA replication (Smith and Stillman 1989).

Knockdown experiments of WSTF and Brg1/hBrm expression via RNA interference in MCF7 cells has shown that WINAC may have a role in chromatin assembly. Depletion of WSTF or Brg1/hBrm by RNAi resulted in altered cell cycle progression, most notably lowered DNA synthesis, suggesting a role for WINAC in replication (Kitagawa et al. 2003).

Pull down assays in cultured MCF7 cells reveal an interaction between WSTF and both ligand-bound and unbound vitamin D receptor (VDR). Chromatin immuno-precipitation experiments in MCF7 cells treated with RNAi specific for WSTF reveal a significant reduction of VDR present at vitamin D-regulated promoters (Kitagawa et al. 2003). Furthermore, decreased VDR recruitment to VDR target genes observed in WS patient skin fibroblasts is rescued by WSTF overexpression. This rescue is likely to be the result of WSTF operating in the context of the WINAC complex, as experiments with MCF7 cells show WINAC-facilitated nucleosome disruption that is VDR-specific (Kitagawa et al. 2003).

VDR binds hormonally-active vitamin D; however, association of WSTF and VDR occurs in a ligand-independent fashion. Given the infantile hypercalcaemia seen in WS patients (and the known links between vitamin D and calcium regulation), researchers have investigated the role of the WINAC complex in vitamin D homeostasis. Two genes important in vitamin D metabolism are hydroxylases 1α (OH)ase and 24(OH)ase (Bouillon et al. 1995). Vitamin D is obtained both from diet, and in the skin in a UV–dependent synthesis. However, newly synthesized vitamin D exists in an inactive hydroxylated form, 25-hydroxyvitamin D (25-OHD) (Norman 1998). The gene 25-hydroxyvitamin D-1α-hydroxylase (1α(OH)ase) is the enzyme responsible for the formation of active vitamin D (1,25-dihydroxyvitamin D). 24(OH)ase (25-hydroxyvitamin D-24-hydroxylase) is the

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enzyme responsible for vitamin D inactivation (Zehnder et al. 2001). WINAC activity facilitates ligand-dependent activation of 24(OH)ase and repression 1α(OH)ase by VDR in MCF7 cells (Figure 4A; Kitagawa et al. 2003). Repression of 1α(OH)ase occurs via a co-regulator swap, from histone acetyltransferase activation complex p300/CBP to a histone deacetylase. This switch occurs on the vitamin D interacting repressor (VDIR), which is constitutively bound to the 1a(OH)ase vitamin D response element (VDRE) (Figure 4B; Murayama et al. 2004).

Researchers have shown in cultured MCF7 cells that a WSTFΔC mutant, which is missing the WAKZ, PHD and bromodomain, still possesses the ability to associate with VDR and other components of WINAC (Kato et al. 2007). However, the WSTFΔC mutant is unable to bind acetylated lysine 14 on histone 3 (AcH3K14), a bromodomain target. Therefore, the WSTFΔC mutant's lack of chromatin interaction is thought to be due to the missing bromodomain, though a role for methyl-lysine binding has not been ruled out. WINAC is thought to facilitate the change of co-regulators on the 1a(OH)ase gene promoter via its ligand-independent binding of VDR and targeting of VDIR even in absence of active vitamin D (Kato et al. 2007). WINAC and VDR are able to locate VDRE through VDR and VDIR unligated interactions and remain at these sites through the associations of the WSTF bromodomain with acetylated histones. Once the VDR binds active vitamin D, the WINAC-VDR complex is already in position to facilitate repression of 1α(OH), which reduces the amount of active vitamin D in the cell (Kato et al. 2007). Future studies will seek to directly link the effects of loss of WSTF on VDR-dependent transcription and defects in calcium regulation in WS patients.

B-WICH

WSTF and ISWI have also found in a complex consisting of eight subunits designated B-WICH (Figure 2B). The B-WICH complex includes Myb-binding protein-1a (Myb-bp1a), Sf3b155/SAP155, Gu alpha, CSB, the proto-oncogene Dek, and nuclear myosin 1. B-WICH also includes several RNAs: 5S rRNA, 7SL RNA, and 45S rRNA (Cavellan et al. 2006). Myb-bp1a is a nucleolar protein known mainly for its role in transcriptional regulation. However, recent studies have identified Myb-bp1a as a substrate for the mitotic enzyme aurora kinase B. RNAi silencing of Myb-bp1a causes mitotic delay and aberrant spindle formation (Perrera et al.). SAP155 is a subunit known to be a part of the spliceosome complex U2 snRNP (Kramer et al. 1999). Gu-alpha is an RNA helicase involved in the processing of ribosomal RNA (Yang et al. 2005). The CSB (**C**ockayne **s**yndrome protein **B**) is a known subunit of another ATP-dependent chromatin remodeling complex (SWI/SNF). CSB appears to bind DNA directly and is crucial for transcription-coupled DNA repair (Beerens et al. 2005). Dek is another DNA binding protein that is thought to function much like an architectural protein in chromatin (Waldmann et al. 2004). NM1 (Nuclear myosin 1) is known to be important for transcription regulation (Percipalle and Farrants 2006; Percipalle et al. 2006).

Many of the B-WICH subunits are known to play a role in gene expression and formation of B-WICH appears to correlate with active transcription (Cavellan et al. 2006). Components of B-WICH (WSTF, ISWI and NM1) are detected at RNA pol I and III genes in HeLa cells by ChIP, suggesting that the B-WICH complex might have a role in nucleosome remodeling to facilitate pol I and III transcription (Cavellan et al. 2006; Percipalle and Farrants 2006), though the specific role of B-WICH has not been elucidated. Research that addresses chromatin conformation and promoter activity are necessary to strengthen the argument for B-WICH-mediated ribosome gene activation. Also, B-WICH localization is not restricted to ribosomal gene promoters, but rather along the entire gene, suggesting that B-WICH may function in more that just activation (Percipalle and Farrants 2006).

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WICH

WICH in DNA replication—WSTF is one of two subunits that comprise the Imitation Switch (ISWI)-containing remodeling complex WICH (Figure 2C). This ISWI complex was first purified from *Xenopus* egg extracts, and is conserved in all vertebrates. In NIH3T3 mouse cells WSTF is concentrated at the heterochromatin around the centromere during replication and prevents aberrant heterochromatin spreading (Bozhenok et al. 2002). The WICH complex also associates with the DNA clamp, PCNA (**P**roliferating-**c**ell **n**uclear **a**ntigen) in HeLa cells. This interaction with PCNA facilitates WICH targeting to replication foci. However, DNA polymerase is still able to elongate in the absence of ISWI, suggesting a role for WICH-dependent chromatin formation after replication (MacCallum et al. 2002; Poot et al. 2004).

WICH and DNA repair

As noted above, recent work has identified an unconventional tyrosine kinase activity present in a region of WSTF encompassing the WAC domain and an additional N-terminal domain (Xiao et al. 2009). WSTF phosphorylates tyrosine 142 of histone H2A.X, and is involved in the DNA damage response. Interestingly, Y142 phosphorylation appears to exist before DNA damage and decreases in response to damage. Y142 phosphorylation is diminished when WSTF is knocked down via RNAi in MEF cells, but S139 phosphorylation (i.e. γ-H2A.X formation) still occurs in response to damage in the WSTF knockdown. However, although S139 phosphorylation occurs normally, phosphorylation and γ-H2A.X focus formation are not maintained in these WSTF-deficient MEF cells. Furthermore, the presence of Mdc1, a nuclear protein with the critical role of sustaining ATM recruitment and thus maintaining γ–H2A.X phosphorylation is diminished in WSTF RNAi-treated cells. It appears that WSTF plays an important role in maintaining S139 phosphorylation and therefore γ–H2A.X focus formation. S139 and Y142 are found in the conserved H2A.X C-terminal motif SQEY, in which S139 is phosphorylated by ATM/ATR. The corresponding motif in budding yeast is SQEL, in which the serine is phosphorylated by the yeast ATM/ATR homologs Tel1 and Mec1. Surprisingly, when the leucine residue in yeast H2A is replaced by a tyrosine, the yeast cells are able to phosphorylate the introduced tyrosine (Xiao et al. 2009), though the responsible kinase has not been identified.

Soon after the novel kinase activity of WSTF was discovered, EYA3 (Eyes Absent 3) was shown to dephosphoryate Y142 on H2A.X in human bone osteosarcoma cells (U2OS) in response to DNA damage (Krishnan et al. 2009). Similarly, EYA1 and 3 were shown to dephosphorylate Y142 in 293T human embryonic kidney cells (Cook et al. 2009). EYA proteins are transcription factors known to play developmental roles in a number of organisms (four, EYA1–4, are present in mammals). EYA proteins are the first transcription factors to also display phosphatase activity (Tootle et al. 2003). Both EYA2 and EYA3 show H2A.X Y142 specific binding and dephosphorylation *in vitro*. Reduction of EYA3 levels by RNAi results in both an increased level of initial tyrosine phosphorylation as well as reduced active dephosphorylation of Y142 following induction of DNA damage *in vivo* (Krishnan et al. 2009). Both EYA1 and EYA3 interact with γ-H2A.X following DNA damage in 293T cells, and are localized to γ-H2A.X foci after damage (Cook et al. 2009). *Eya1*$^{-/-}$ knockout mice exhibit increased apoptosis in the developing kidney at embryonic day 11.5, and EYA1 or EYA3 knockdowns in 293T cells also result in increased apoptosis in response to hypoxia. Interestingly, EYA3 is phosphorylated by ATM/ATR, and this appears to be required for EYA3-H2A.X interaction. These authors also showed that the pro-apoptotic kinase JNK1 interacts with S-Y biphosphorylated peptides, but not with singly phosphorylated peptides. These studies suggest a possible role for Y142 de/phosphorylation in the balance between repair and apoptosis.

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Taken together, these discoveries reveal a complex interaction in which WSTF kinase activity targets H2A.X, providing a novel role for the WICH complex in the DNA damage response, while identifying the phosphoY142 phosphatase, EYA implicated in apoptotic pathways (the major interactions are depicted in Figure 5) (Cook et al. 2009; Krishnan et al. 2009; Xiao et al. 2009). H2A.X phosphoY142 appears to be globally dephosphorylated in response to DNA damage as antibodies are unable to detect phosphoY142 8 hours after cell irradiation (Xiao et al. 2009). This result seems contradictory to studies that show EYA acting locally, dephosphorylating H2A.X phosphoY142 at sites of DNA damage. It will be important for future studies to investigate whether H2A.X phosphoY142 exists globally or in particular areas of chromatin and to confirm that H2A.X phosphoY142 is dephosphorylated throughout the genome by EYA.

Conclusion

WSTF is a member of three known chromatin remodeling complexes: WINAC, WICH and B-WICH. The gene *WBSCR* that codes for WSTF is one of a number of genes within a heterozygous deletion that exists in individuals with Williams Syndrome. WSTF functions in a surprising number of different roles in a diverse set of chromatin remodeling complexes. A great deal of new information has been obtained since WSTF was first identified, yet many questions about the role of WSTF in replication, gene regulation and DNA repair—and in the clinical presentations of WS patients—still remain. However, a narrative is beginning to build as more research continues to address the questions surrounding this versatile chromatin modifying factor.

Acknowledgments

We thank Shannon Uffenbeck and Oya Yazgan for critical reading of the manuscript and assistance with the figures. Work in our laboratory on WSTF is supported by The Whitehall Foundation #2007-08-79, NIH/NEI #EY016029-02, and Alaska INBRE, NIH/NCRR #RR016466.

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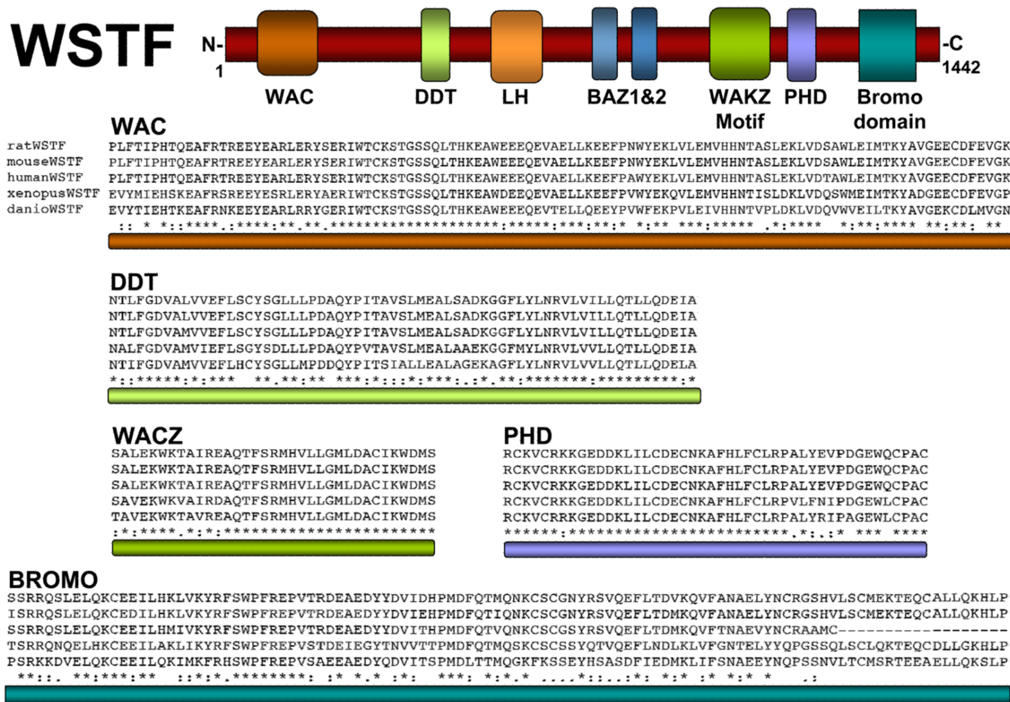

Figure 1.

Conserved motifs in WSTF. Top: schematic diagram of WSTF depicting the relative location of conserved protein domains LH, WAC, DDT, BAZ1, BAZ2, WAKZ, PHD and bromodomain. Bottom: sequence alignments of the specified domains illustrate the high degree of conservation among multiple species. “*” the residues or nucleotides in that column are identical in all sequences in the alignment; “: “ conserved substitutions have been observed; “.” semi-conserved substitutions are observed.

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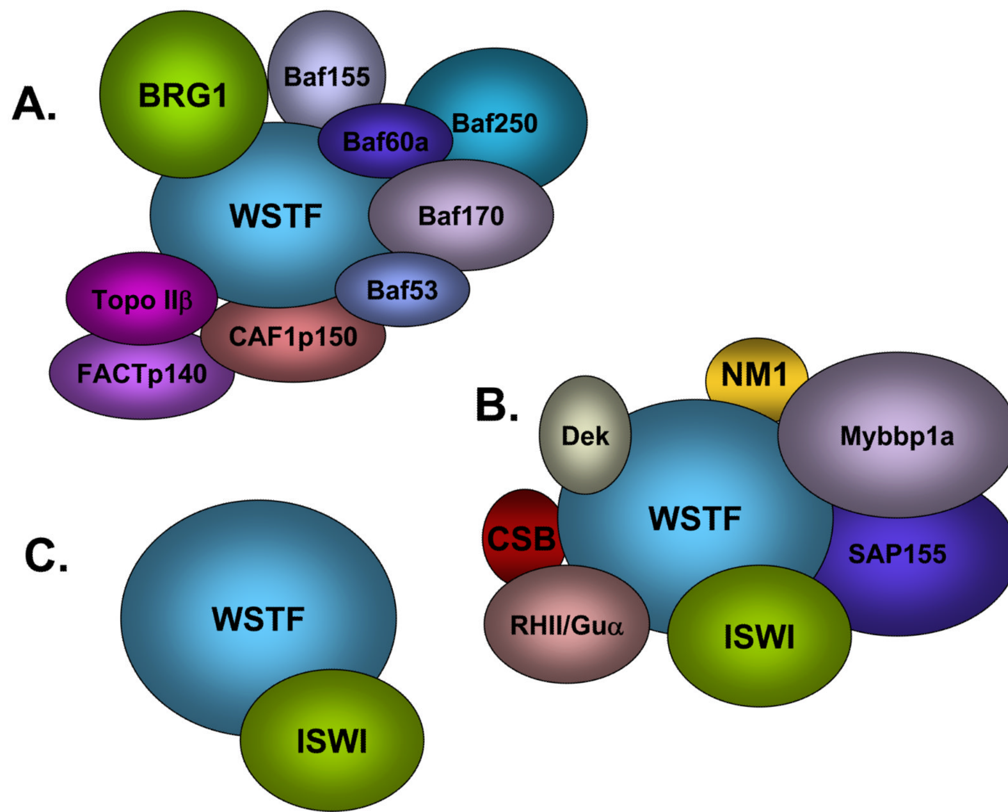

Figure 2.

WSTF-containing chromatin remodeling complexes. (A) WINAC. WINAC contains SWI/SNF ATPases Brg1 and Brm, 5 BAF (Brg-1 associated factor) subunits (BAF53, 60, 70, 155 and 250), TopoIIβ, FACT p140, CAF1 p150 and WSTF. (B) B-WICH. The B-WICH complex includes Myb-binding protein-1a (Myb-bp1a), Sf3b155/SAP155, Gu alpha, CSB, the proto-oncogene Dek, and nuclear myosin 1. B-WICH also includes several ribosomal RNAs 5 S rRNA, 7SL RNA, and 45 S rRNA. (C) WICH. WICH complex contains WSTF (Williams Syndrome Transcription Factor) and ISWI (Imitation Switch).

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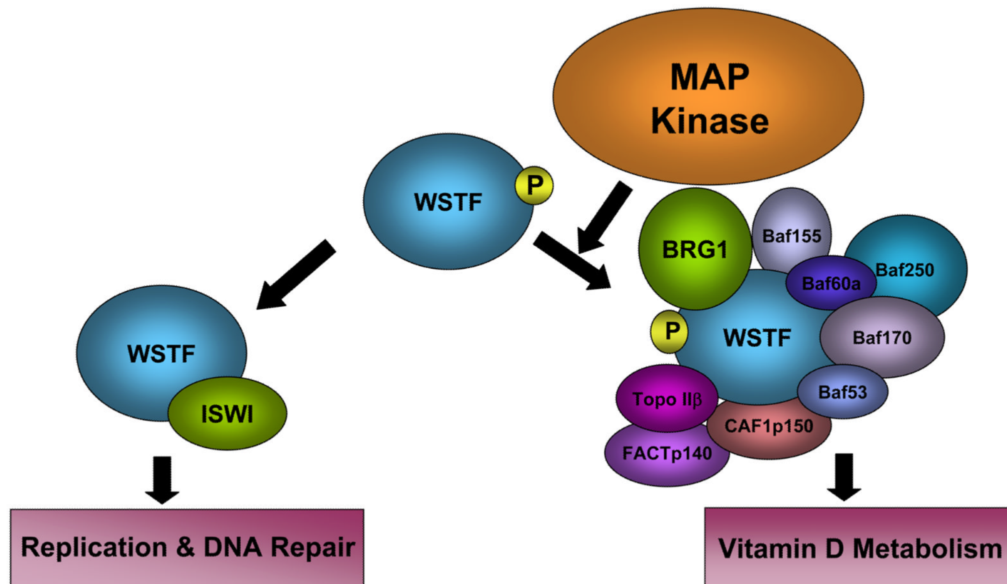

Figure 3.

MAPK targets WSTF. A schematic representation of MAP kinase phosphorylation of WSTF. MAP kinase phosphorylates WSTF on Serine 158 within the WAC domain. WSTF phosphorylation is important for WSTF integration into the WINAC complex, but appears to be dispensable for assembly of the WICH complex.

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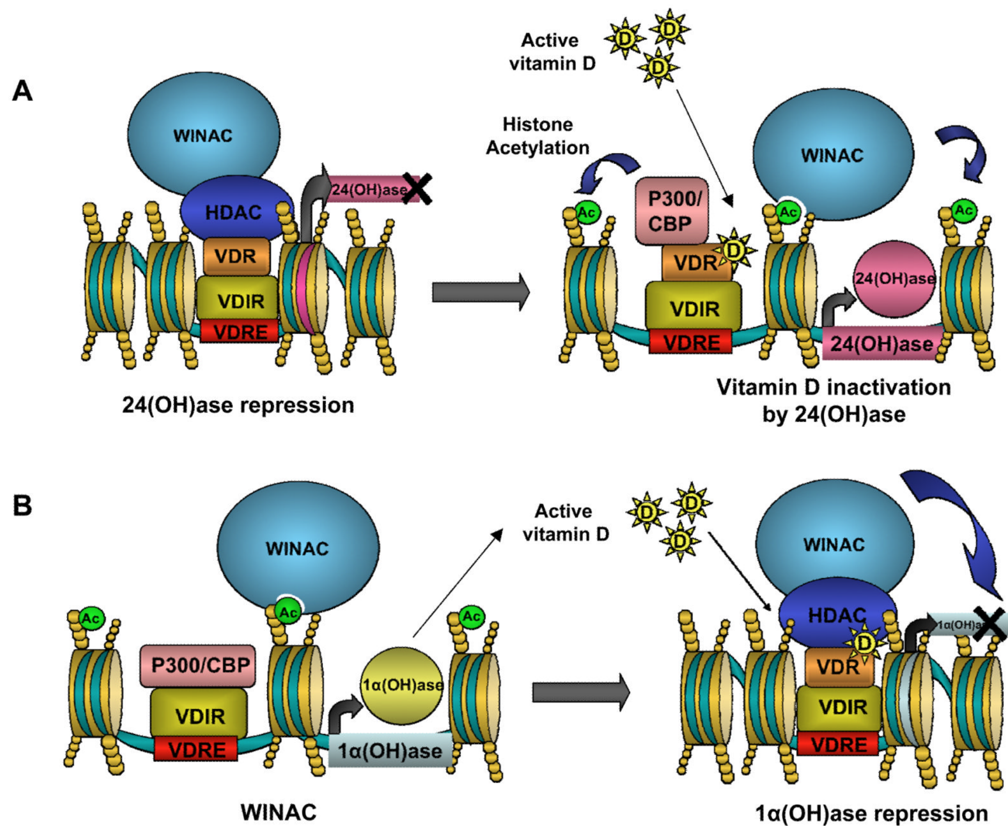

Figure 4.

WINAC and VDR-dependent transcription regulation. (A)WINAC-VDR complex facilitates activation of 24(OH)ase. In the absence of vitamin D, unliganded VDR is bound to the VDIR along with transcriptional repressing histone deactylase. Once VDR binds active vitamin D it facilitates a co-regulator swap from histone deacetylase to histone acetyltransferase activation complex p300/CBP. The result is activation of 24(OH)ase by WINAC chromatin remodeling. (B) WINAC-VDR complex is already in position to facilitate repression of 1a(OH). WINAC and VDR are able to locate VDRE through VDR and VDIR unliganded interactions and remain at these sites through the association of the WSTF bromodomain with acetylated histones. Once VDR binds vitamin D the VDIR experiences a co-regulator swap, from histone acetyltransferase activation complex p300/CBP to a histone deacetylase. The result is repression of 1a(OH)ase by WINAC chromatin remodeling.

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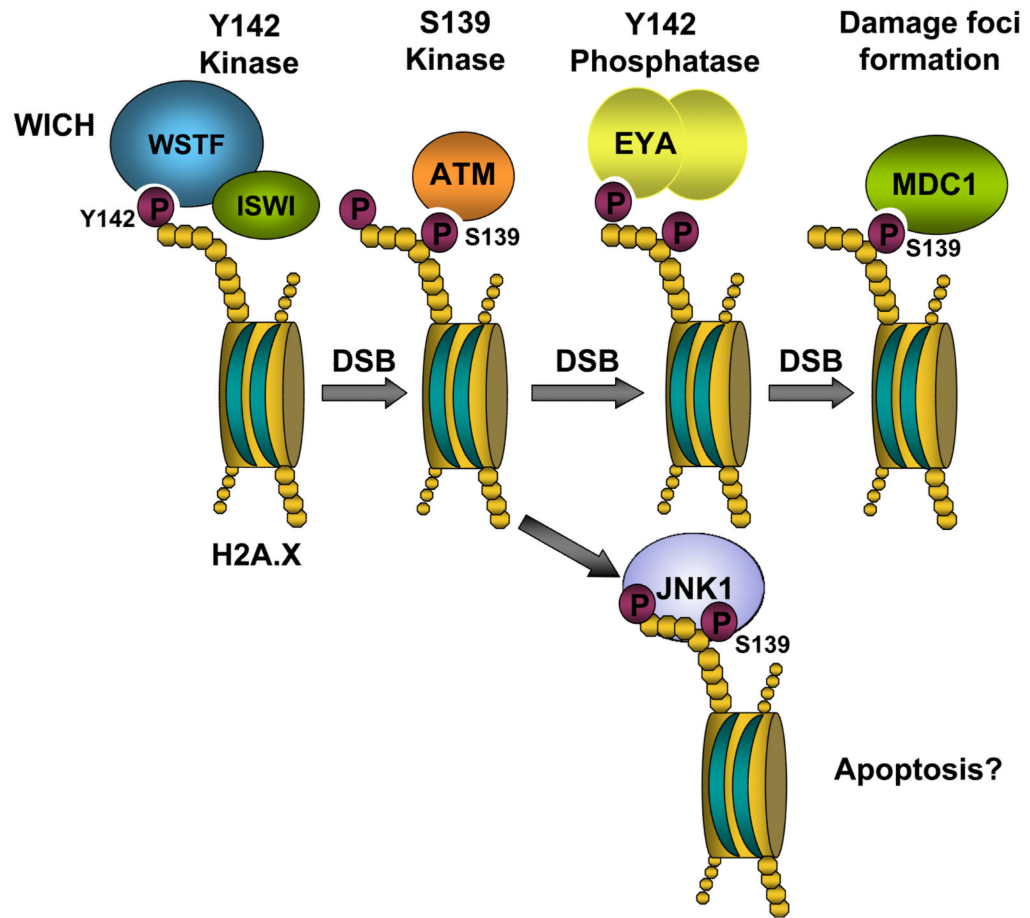

Figure 5.

The WICH complex has a role in DNA repair. The WICH complex is a kinase, targeting tyrosine 142 on histone H2A.X. Y142 is phosphorylated before damage occurs and becomes gradually dephosphorylated after DNA damage by EYA. MDC1 binds phosphorylated S139 and facilitates foci formation.