The chromatin-remodeling complexes B-WICH and NuRD regulate ribosomal transcription in response to glucose

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Abstract
Regulation of ribosomal transcription is under tight control from environmental stimuli, and this control involves changes in the chromatin structure. The underlying mechanism of how chromatin changes in response to nutrient and energy supply in the cell is still unclear. The chromatin-remodeling complex B-WICH is involved in activating the ribosomal transcription, and we show here that knock down of the B-WICH component WSTF results in cells that do not respond to glucose. The promoter is less accessible, and RNA pol I and its transcription factors SL1/TIF-1B and RRN3/TIF-1A, as well as the proto-oncogene c-MYC and the activating deacetylase SIRT7 do not bind upon glucose stimulation. In contrast, the repressive chromatin state that forms after glucose deprivation is reversible, and RNA pol I factors are recruited. WSTF knock down results in an accumulation of the ATPase CHD4, a component of the NuRD chromatin remodeling complex, which is responsible for establishing a repressive poised state at the promoter. The TTF-1, which binds and affect the binding of the chromatin complexes, is important to control the association of activating chromatin component UBF. We suggest that B-WICH is required to allow for a shift to an active chromatin state upon environmental stimulation, by counteracting the repressive state induced by the NuRD complex.

KEYWORDS
CHD4, chromatin remodeling, c-MYC, ribosomal genes, TTF-1, WSTF

Abbreviations: AMPK, AMP-kinase; CHD4, chromodomain-helicase-DNA binding protein 4 (aka Mi-2β); CSB, Cockayne syndrome protein B; DMNT, DNA methyl transferase; eNOSc, energy-dependent nucleolar silencing complex; HAT, histone acetyl-transferase; HDAC, histone deacetylase; MAPK, mitogen-activated protein kinase; NM1, nuclear myosin 1; NoRC, nucleolar remodeling complex; NuRD, nucleosome Remodeling Deacetylase; P3K, phosphatidylinositol 3-kinase; RB, retinoblastoma protein; RRN3/TIF-1A, transcription initiation factor 1A; SIRT1, Siruin 1; SL1/TIF-1B, selective factor 1/Transcription initiation factor 1B; SNF2h, sucrose non-fermenting protein 2 homolog; TBP, TATA box binding protein; TIP5, TTF-1 interacting protein 5; TTF-1, transcription terminator factor 1; UBF, upstream binding factor; WSTF, Williams syndrome transcription factor.

Anna Rolicka and Yuan Guo contributed equally to this study.

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1 | INTRODUCTION

Ribosomal biogenesis is one of the major consumers of energy in proliferating cells in order to meet the requirement of ribosomes for protein synthesis. Therefore, transcription of ribosomal RNA (rRNA) genes and ribosomal protein genes is regulated in response to the metabolic state in cells (reviewed in 1,2). Transcription of the ribosomal gene repeats in the nucleolus constitute the most highly transcribed genes and employ their own RNA polymerase I (RNA pol I) machinery. The ribosomal protein genes and the fourth type of ribosomal gene, the SS rRNA genes, are transcribed by RNA pol II and RNA pol III, respectively, subjecting these genes to a strong need for co-ordination. Several signaling pathways responding to the nutrient and energy status as well as to proliferative signals and stressors are regulating the transcription of all type of ribosomal genes simultaneously, and dysregulation often lead to senescence or apoptosis. However, the mechanisms of how the individual type of gene is regulated is not fully understood.

Signaling pathways regulate RNA pol I ribosomal transcription mechanistically on many levels: by modification of transcription factors, altering chromatin changes, and inducing noncoding RNA. Activation of general signaling pathways, such as mTOR, PI3K, and the MAPK pathways, leads to phosphorylation of the components in the RNA Pol I machinery, such as RRN3/TIF-1A, the promoter proteins SL1/TIF-1B, and the upstream binding factor UBF.3,4 The posttranslational modifications change the interactions between the proteins and the promoter.5-7 Transcription factors, such as c-MYC, RB, and p53, also regulate rRNA gene expression by associating either directly with DNA or binding to SL1/TIF-1B and UBF and regulating their activity.2,4,6,7 Such factors are also responsible for changing the chromatin state at the rRNA genes; they recruit enzymes that change the patterns of histone modifications and DNA methylation by targeting histone acetyl transferases (HATs), histone deacetylases (HDACs), histone methyl transferases, and DNA methylases (DNMTs) to the promoter.8,9 In addition, the chromatin structure at the rRNA gene promoter is altered by chromatin-remodeling complexes and chromatin protein that are recruited by transcription factors.8,9 rRNA gene transcription is also regulated by higher order chromatin structures, such as chromatin loops formed by TTF-110 and c-MYC11,12 are required for full transcription and TIP5-NoRC for silencing.13

Ribosomal RNA genes are present in multiple copies and the diploid human genome contains approximately 400 rRNA gene copies at several loci. Not all genes are active in differentiated cells, and activation can either increase the initiation and elongation rates of already active genes, or remodel silent genes.4,9,14 Active copies are characterized by the association of UBF at the upstream region, at the promoter, and in the gene body region, which results in a decondenced chromatin configuration.4,14-16 The silent copies are organized in heterochromatin and a number of silencing chromatin remodeling complexes are associated with rDNA. The NoRC complex silences rRNA gene copies by nucleosome remodeling, silent histone modification, such as H3K9me2/3 and DNA methylation.17-19 Other silencing chromatin-remodeling complexes confer a pseudo-silent state or poised chromatin state. The NuRD complex, comprising the ATPase CHD4, the methyl binding protein MBP1 and the two histone deacetylases HDAC1 and HDAC2, establishes a poised state at rRNA genes characterized by a positioned nucleosome over the transcription start site in the promoter.20-23 The binding of histone H1, together with HP1, at the promoter also silences transcription.4,24 The eNoSC complex, composed of nucleomethylin (NML), the histone deacetylase SIRT1, and the histone methyl transferase SUV39H1, forms a repressive state at the promoter specifically in response to a low energy status25-27 or stress, causing senescence.28 The silent states, mainly the poised or pseudo-silent state, may be activated by external stimuli but the mechanism of the switch is poorly understood.

Activating factors, both transcription factors and chromatin-remodeling complexes, have been identified in addition to chromatin factor UBF. Two ATP-dependent chromatin-remodeling factors, CSB (Cockayne syndrome protein B)29-31 and B-WICH,32,33 have been associated with the activation of rRNA genes by changing the chromatin structure. CSB, which is an ATPase, reverses the poised state induced by the NuRD complex20 and the NoRC complex,34 by moving a nucleosome away from the transcription start site in the promoter. B-WICH is composed of Williams Syndrome Transcription Factor (WSTF), the ATPase SNF2h, and nuclear myosin 1 (NM1).35 Knock down studies of WSTF have demonstrated that B-WICH modulates the chromatin structure over the rRNA gene promoter to allow HATs to bind and increase the acetylation of histone H3.35 B-WICH is involved in the activation of rRNA gene transcription together with c-MYC in hypertrophic muscle, where it is expressed to a higher level and associates with the rRNA gene promoter.36 The CSB and the B-WICH may also co-operate, since CSB interacts with the WSTF protein in the B-WICH complex.33 However, it is not clear whether B-WICH is involved in the switch from an inactive to an active state upon environmental cues.

In order to study mechanisms by which RNA pol I gene transcription are activated in the response to environmental cues, we focused on how B-WICH activates transcription in response to glucose stimulation. The rates of cell growth and proliferation are directly related to ribosomal biogenesis and they are, therefore, tightly regulated by nutrient supply.2,37 rRNA genes respond to low energy levels in the cells by using two different repressive mechanisms; in addition to the action of the eNoSC complex,25 the AMPK signaling pathway controls the level of rRNA gene transcription by inactivating RRN3/TIF1-A
by inducing a specific, negative phosphorylation. Here, we show that chromatin changes by B-WICH are necessary for the recruitment of the RNA Pol I machinery and activating factors, such as c-MYC, upon activation of transcription in response to glucose. The closed chromatin configuration is not induced by the eNoSC; instead the NuRD ATPase CHD4 is not released from the promoter upon glucose stimulation in the absence of WSTF. We propose that the B-WICH acts by establishing an active permissive chromatin configuration by switching the repressive poised state that is formed by the CHD4-NuRD complex by releasing the CHD4. This switch is required to obtain basal transcription of the RNA pol ribosomal genes. Furthermore, the B-WICH and NuRD also stabilize TTF-1 (Transcriptional terminator factor-1) and regulate the binding of c-MYC to the promoter, both proteins involved in higher order chromatin structures required for full transcription.

2 | MATERIALS AND METHODS

2.1 | Cell lines and treatments

Adherent HeLa cells and HEK293 cells were purchased from ATCC, USA, and the HeLa cells were kept for a maximum of 30 passages. The HeLa and HEK cells were cultivated at 10% of CO2 in DMEM high-glucose medium (4.5 g/L) supplemented with 10% of Fetal Bovine Serum and Penicillin/Streptomycin. Glucose-deprived cells were grown in DMEM medium without glucose for 18 hours, and then, re-fed with DMEM high-glucose (4.5 g/L) medium for six hours if not stated otherwise.

2.2 | Antibodies

Antibodies against WSTF, SNF2h, histone H3, H3K9-Ac, H3K14-Ac, histone H1, RNR3 (TIF-1A), PCAF, GCN5, p300, SIRT1, HDAC2, CHD4, TBP, TAF1C, TAF1B, fibrillarin, and IgG were from Abcam. Antibodies against UBF and c-MYC were from Santa Cruz and Abcam, SIRT7 from Sigma, TTF-1 from Gentex and antibodies RNA Pol I were a kind gift from Dr T. Moss, from Santa Cruz and Aviva Systems Biology.

2.3 | Transfections

HeLa cells and HEK293 cells were transfected using RNAiMAX (Invitrogen), a combination of two siRNAs targeting WSTF, CHD4, and TTF-1 each, and a control siRNA (Eurofins) were used. The incubations were typically for 18-24 hours before glucose starvation and glucose stimulation. The sequence of the two siRNAs against each mRNA are given in Table S1, and designed via the designer tool provided by Eurofins. One siRNA for WSTF was used also in used in Vintermist et al, 2011, Sarshad et al, 2013, and Sadeghifar et al, 2015.

2.4 | RNA extraction and cDNA synthesis

Cells were lysed by Tri lysis reagent (Ambion) according to the manufacturer’s instructions. The RNA was converted to DNA by Superscript III (Invitrogen) using random primers (Invitrogen). The samples were analyzed using the primer pairs presented in Table S2. The human sequence used; GenBank Accession No. U13369.

2.5 | Chromatin immunoprecipitations (ChIP)

ChIP analyses were performed as described in Vintermist et al. on cross-linked chromatin (1% of formaldehyde) from different treatments. The IP was performed in 1% of Triton X-100 and 0.1% of DOC, to which Protein A/G Sepharose (GE Health care) was added. qPCR was performed using SYBR-green from KAPA (Merck/Sigma Aldrich) according to the manufacturer’s instructions. The PCR conditions were: hold 95°C for 3 minutes, followed by cycles of 95°C for 3 seconds, 60°C for 20 seconds, 72°C for 3 seconds. The results are presented as percentage of input, with the no Ab control and IgG control subtracted from the Ct values obtained in the samples precipitated with specific antibodies. The primer pairs used to produce amplicons are presented in Table S2.

2.6 | MNase digestion assay

MNase assays were performed as described in Vintermist et al. Briefly, cross-linked chromatin (1%) was sonicated briefly prior to treatment. The chromatin was of the same concentration, measured by OD280 and the amount of MNase titrated for each experiment. The digested DNA was evaluated by qPCR, and analyzed by calculating ΔCt between the reaction with no MNase and the digested reaction at each concentration. The primer pairs used for MNase digestion over the rRNA gene promoter are presented in Table S2.

2.7 | Extract preparation, immunoprecipitation and SDS-PAGE

Nuclear extract (0.7 M and 0.42 M NaCl) and cell extract (0.7 M NaCl) from HeLa cells were prepared, and the
proteins separated on a 7% or 10% of SDS-PAGE prior to transfer to a PVDF membrane (Millipore). Extracts for immunoprecipitations were diluted to 0.35 M NaCl before the incubation with specific antibodies. Proteins-antibody complexes were precipitated using Protein A/G beads. The quantifications of the signals were performed using the Bio-Rad GelDoc Quantification program.

2.8 | Immunofluorescence

HeLa cells were seeded on coverslips and exposed to siRNAs for WSTF or a control siRNA for 24 hours. The cells were fixed with 3.7% of formaldehyde, permeabilized with 0.1% of Triton X-100 in PBS and blocked with 3% of BSA. The cells were stained with anti-fibrillarin antibody and DAPI.

3 | RESULTS

3.1 | WSTF is required for activated rRNA transcription upon glucose stimulation

B-WICH is involved in active ribosomal transcription and is recruited to the rRNA gene promoter upon transcriptional activation after serum starvation. rRNA transcription is also regulated by the glucose level and to investigate whether the B-WICH activity is involved in the response to nutrients, we examined the activation upon glucose stimulation in HeLa cells. The level of rRNA was almost abolished after 18 hours of glucose deprivation and refeeding using a medium containing 4.5 g/L of glucose gave a strong increase after 6 hours before returning to the same level as in exponentially growing cells at 12 hours (Figure S1A). WSTF knock down in cells grown in medium containing 4.5 g/L of glucose exhibited a low level of rRNA transcription (Figure 1A), and transcription was not resumed in glucose stimulated cells after 18 hours of glucose deprivation as it did in cells transfected with a control siRNA (Figure 1A). The knock down of WSTF by the two siRNAs is shown in Figure S1B.

3.2 | B-WICH is required for factor loading upon glucose stimulation

The lack of response in WSTF knock down cells (WSTF KD cells) to glucose stimulation prompted us to investigate the binding of RNA Pol I and general transcription factors to the rRNA gene promoter in response to glucose using chromatin immunoprecipitation (ChIP). Similar to serum starvation, the association of WSTF with the promoter was low in glucose starved cells, and higher when glucose was reintroduced for 6 hours (Figure 1B). This pattern of association of WSTF was also observed in HEK293 cells (Figure S1C). Glucose deprivation resulted in a low level of the RNA Pol I associated with the promoter and it increased upon glucose stimulation in control cells. This increase upon glucose stimulation was not observed in WSTF KD cells (Figure 1B). The level of UBF associated with the rRNA gene promoter, in contrast, remained the same regardless of treatment (Figure 1B). RNA Pol I recruitment to the promoter requires the RRN3/TIF-1A and the platform formed by the SL1/TIF-1B complex in mammalian cells. Therefore, we investigated the binding of the RRN3/TIF-1A and the SL1/TIF-1B proteins TBP, TAF1B, and TAF1C to the promoter. The binding of all these factors was abolished in glucose starved cells and elevated after glucose refeeding in control cells (Figure 1C). In WSTF KD cells, the association of RRN3/TIF-1A, TBP, TAF1B, and TAF1C to the promoter was low in glucose deprived cells and they failed to bind when glucose was reintroduced (Figure 1C). We also controlled for RNA pol I and RRN3/TIF-1A binding in the intergenic region at position 27 Kb from the start site and no association could be detected (27 kb in Figure 1B,C). We conclude that the binding of RNA pol I, RRN3/TIF-1A, and SL1/TIF-1B to the promoter is impaired in WSTF KD cells.

In order to examine the integrity of the nucleolus in WSTF KD cells, we performed immunofluorescence with fibrillarin, an rRNA processing protein. WSTF knock down induced a redistribution of fibrillarin, which exhibited nucleolar ring and cap formations indicative of a stressed response (Figure S1D). These structures were rare in exponentially growing cells, but increased when the cells were depleted of glucose for 18 hours (Figure S1E). Glucose depletion did not change the fraction of stress nucleoli in WSTF KD cells (Figure S1E). The redistribution of fibrillarin is not caused by a changed protein level by WSTF knock down or by glucose depletion, at least not in WSTF KD cells since the fibrillarin level was higher (Figure S1F).

3.3 | B-WICH is required for the response after both long-term and short-term deprivation

Long-term deprivation of nutrients or growth factors, such as serum deprivation of fibroblasts for 18 hours, may cause cells to enter G0/G1 cell-cycle states or induce a stress situation, after which de novo initiation of transcription is rate-limiting. In order to examine whether B-WICH was required also for induction of transcription after short-term glucose deprivation, we investigated rRNA levels and factor recruitment after only 2 hours glucose deprivation followed by...
6 hours glucose stimulation. The level of rRNA after 2 hours was downregulated in control cells, but to a lesser extent than 18 hours depletion, and the level increased upon glucose stimulation for 6 hours, not to an enhanced level but to the same level as the one found in exponentially growing cells (Figure 1D). Similar to 18 hours of starvation, WSTF KD cells exhibited a low level of rRNA after 2 hours of starvation and the level did not increase when glucose was reintroduced (Figure 1D). We next investigated the association of the factors with the promoter and in contrast to being nearly absent at the promoter in 18 hours starved cell, the general RNA pol I factors associated to a low level in both control cells and in WSTF KD cells after 2 hours glucose depletion. Nevertheless, factor recruitment of RNA pol I, RRN3/TIF-1A, and SL1/
FIGURE 1  rRNA transcription is increased upon glucose stimulation in control cells, but impaired in WSTF KD cells. A, qPCR analysis of rRNA gene transcription levels in HeLa cells transfected with siRNAs against WSTF or a control siRNA. The cells were grown in normal growth conditions, deprived of glucose for 18 hours (−), or stimulated with glucose for six hours after 18 hours of glucose deprivation (+). Results are normalized to cells under normal growth conditions. N = 6. mean ± SD (normal: control vs WSTF siRNA *P = 1 × 10−6; control siRNA *P = .00006, WSTF siRNA n.s.; normal control vs control −glc *P = 0.5 × 10−6, normal control vs +glc *P = .00009). B, qPCR of ChIP analysis of WSTF, RNA Pol I and UBF in cells transfected with siRNAs against WSTF or control siRNA. Cells were glucose deprived for 18 hours (−) or glucose stimulated for six hours after 18 hours of glucose deprivation (+). The values are presented as percentages of the input signal. N = 5. mean ± SD. (WSTF control siRNA *P = .0005, Pol I, control siRNA **P = .005, WSTF siRNA n.s.; UBF. all values n.s.). C, qPCR of ChIP analysis of SL1/TIF-1B components TBP, TAF1B, TAF1C and RRN3/TIF-1A in cells transfected with siRNAs against WSTF or control siRNA. Cells were glucose deprived for 18 hours (−) or glucose stimulated for six hours after 18 hours of glucose deprivation (+). The values are presented as percentages of the input signal. N = 5. Mean ± SD. (RRN3. control siRNA *P = .009, WSTF siRNA n.s.; TBP. Control siRNA *P = .049, WSTF siRNA n.s.; TAF1B, control siRNA *P = .012, WSTF siRNA n.s.; TAF1C. control siRNA *P = .022, WSTF siRNA n.s.). D, qPCR analysis of rRNA gene transcription levels in cells transfected with siRNA against WSTF or a control siRNA. The cells were grown in normal growth conditions, glucose deprived for two hours (−), or glucose stimulated for six hours after two hours of glucose deprivation (+). Results are normalized to control cells under normal growth conditions. N = 7. mean ± SD. Control siRNA *P = .017, WSTF siRNA P = n.s; Normal control vs control −glc **P = .004). E and F, Quantification of Immunoblots of whole cell extract (30 µg protein). Cells transfected with siRNAs against WSTF or control siRNA were glucose deprived for 18 hours (−glc) or glucose stimulated for six hours after 18 hours of glucose deprivation (+glc). The immunoblots were probed with antibodies to (E) RRN3/TIF-1A-P(S649) and (F) RRN3/TIF-1A, tubulin was used as loading control to both antibodies. The antibodies used are marked on the right. The molecular weight marker is depicted on the left. Quantification of immunoblots (upper panels) for (E) RRN3/TIF-1A-P(S649) normalized to tubulin. N = 4. Mean ± SD (control −glc vs WSTF siRNA +glc **P = .015) and (F) RRN3/TIF-1A normalized to tubulin. N = 4. Mean ± SD. (control −glc vs WSTF siRNA +glc **P = .003)

TIF-1B in control cells increased upon glucose stimulation, whereas in glucose-stimulated WSTF KD cells factors remained at the same low level at the promoter as in glucose deprived cells (Figure S1G). Taken together, WSTF knock down prevents de novo recruitment of the transcription machinery, in particular RNA Pol I, to the promoter after both short-term and long-term glucose deprivation. This supports that B-WICH is required to properly switch on rRNA gene transcription at the initiation step. Hence, we choose to glucose deprive cells for 18 hours and stimulate for 6 hours in subsequent experiments.

3.4   The activation of rRNA transcription by B-WICH is not regulate by phosphorylation of RRN3/TIF-1A

The RRN3/TIF-1A is targeted by several signaling pathways both to activate and to repress transcription by phosphorylating different sites on the protein. Phosphorylation of Ser-649 is required for the B-WICH component NM1 to associate with RNA Pol I and actin to activate transcription, but we could not detect a change in the level upon glucose stimulation in control cells (Figure 1E, quantification in the lower panel, n = 4). The level of phosphorylated RRN3/TIF-1A was high in glucose stimulated WSTF KD cells (Figure 1E, quantified in the lower panel). However, this increase followed the increased level of total RRN3/TIF-1A protein (Figure 1F, quantification in the lower panel), giving a ratio for P-S649-RRN3/RRN3 of 0.98. Glucose deprivation for 2 hours showed similar effects, with a trend of having a higher protein level of RRN3/TIF-1A in WSTF KD cells, and the RRN3/TIF-1A was correspondingly phosphorylated at Ser-649 (Figures S1H,I). We conclude that despite an increased level of RRN3/TIF-1A which was phosphorylated on Ser-649 in WSTF KD cells, RNA Pol I and RRN3/TIF-1A could not be recruited to the rRNA promoter upon glucose stimulation. This indicates that WSTF knock down has a structural effect on the promoter.

3.5   B-WICH is required for increased promoter accessibility upon glucose stimulation

The lack of RRN3/TIF-1A recruitment to the promoter despite a higher level of Ser-649 phosphorylation, together with the impaired recruitment of other factors upon glucose stimulation in WSTF KD cells, led us to investigate the chromatin state at the promoter in WSTFKD cells. We have shown that WSTF knock down results in a reduction in histone H3K9Ac at the promoter and a more closed chromatin. Glucose deprivation for 18 hours reduced the histone acetylation levels of H3K9Ac and H3K14Ac, and the levels were induced after glucose stimulation in control cells (Figure 2A). In WSTF KD cells, however, H3K9Ac and H3K14Ac were not induced after glucose stimulation (Figure 2A). The HATs PCAF, GCN5, and P300 were recruited to the promoter upon glucose stimulation in control cells, while the level remained low both in glucose deprived and stimulated WSTF KD cells in (Figure 2B). This suggesting that the B-WICH is required for the recruitment of HATs and the establishment of a promoter structure with more activating histone marks.

WSTF knock down decreases the MNase accessibility of approximately 200 bp at the promoter in exponentially
grown cells, and we next compared the accessibility in control cells with that in WSTF KD cells when these cells were exposed to glucose depletion for 18 hours and subsequent glucose stimulation for 6 hours. Glucose deprivation in control cells resulted in a closed promoter, inaccessible to MNase digestion, but this changed to a more accessible configuration after 6 hours of glucose stimulation (Figure 2C). WSTF KD cells exhibited a closed configuration under both glucose deprivation and glucose stimulation (Figure 2C), further suggesting that the chromatin structure at the promoter requires B-WICH to change to a more open configuration.

3.6 | B-WICH is required for the loading of activating factors upon glucose stimulation

Next, we investigated the impact of the less accessible chromatin at the promoter in WSTF KD cells on the binding of two transcriptional activators, SIRT7 and c-MYC. SIRT7
activates RNA gene transcription by interacting with UBF, WSTF, and SNF2h, and by deacetylating the RNA pol I subunit PAF53 during glucose refeeding. The level of SIRT7 bound to the RNA promoter was reduced upon glucose depletion, and its binding to the promoter was significantly increased after glucose stimulation (Figure 2D). In WSTF KD cells, no increase occurred upon glucose refeeding (Figure 2D).

**3.8 | B-WICH promotes an open chromatin configuration at the RNA gene promoter**

The RNA promoter is regulated by a number of chromatin factors which activate and silence the transcription and next, we investigated the interplay of B-WICH with the silencing factors CHD4 in the NuRD complex and histone H1. The CHD4 in WSTF KD cells accumulated at the promoter compared with exponentially growing control cells (Figure 3B), despite the fact that the protein level was the same (Figure S2A). The accumulation of CHD4 also occurred in WSTF KD exponentially growing HEK cells (Figure S2B), suggesting that the CHD4 in the NuRD complex is dependent on the B-WICH for its release. The association of histone H1 did not exhibit any difference in association between control and WSTF KD cells (Figure 3B).

**FIGURE 2** WSTF KD affects chromatin configuration at the promoter and prevents the binding of activating factors. A, ChIP-qPCR analysis of H3K9-Ac and H3K14-Ac at the rRNA gene promoter in cells transfected with siRNAs against WSTF and control siRNA. Cells were glucose deprived for 18 hours (−) or glucose stimulated for six hours after 18 hours of glucose deprivation (+glc). The levels of H3K9-Ac and H3K14-Ac are related to the level of H3. N = 5. Mean ± SD. B, CHIP-qPCR of PCAF, GCN5 and p300 at the rRNA promoter in cells transfected with siRNAs against WSTF and control siRNA. Cells were glucose deprived for 18 hours (−) or glucose stimulated for six hours after 18 hours of glucose deprivation (+). Antibodies are indicated above the bars. The values are presented as percentages of the input signal. N = 5. Mean ± SD. (p300. control siRNA **P = .0012, WSTF siRNA n.s.; hGCN5. control siRNA **P = .0019, WSTF siRNA n.s.; PCAF. control siRNA **P = .0012, WSTF siRNA n.s.). C, qPCR analysis of chromatin treated with micrococcal nuclease. A schematic diagram of the rRNA gene is drawn above the graph, where the arrow indicates the transcription start site, and primer positions are indicated (base distance from transcription start site). Control cells (full lines) and WSTF KD cells (dashed lines) were grown in no glucose for 18 hours (−glc, black lines) or re-fed with glucose for six hours after 18 hours of glucose deprivation (+glc, red lines). The primer products were 100 bp. The chromatin profile is presented as 2ΔCt of undigested and MNase digested cross-linked chromatin. N = 4. Mean ± SD. E, ChIP-qPCR analysis of c-MYC in cells transfected with siRNAs against WSTF or control siRNA. The region of the rRNA gene is drawn above the graph, where the arrow indicates the transcription start site, and primer positions are given for promoter (light blue) and +1kb (dark blue) regions. Cells were glucose deprived for 18 hours (−) or glucose stimulated for six hours after 18 hours of glucose deprivation (+). The values are presented as percentages of the input signal. N = 3. mean ± SD. (Promoter: control siRNA *P = .015, WSTF siRNA n.s.; +1kb. control siRNA *P = .03, WSTF siRNA n.s.). D, ChIP-qPCR analysis of SIRT7 at the rRNA gene promoter in cells transfected with siRNAs against WSTF or control siRNA. Cells were glucose deprived for 18 hours (−) or glucose stimulated for six hours after 18 hours of glucose deprivation (+). The values are presented as percentages of the input signal. N = 3. mean ± SD. (control siRNA *P = .017, WSTF siRNA n.s.)
To investigate whether NuRD and histone H1 work with the B-WICH in the response to glucose stimulations, we examined the binding of these factors at different glucose concentrations. The CHD4 associated with the promoter under glucose deprivation in both control cells and WSTF KD cells (Figure 3C). However, while CHD4 was released upon glucose stimulation in control cells, it remained associated with the promoter in WSTF KD cells (Figure 3C). In contrast, histone H1 dissociated from the promoter after glucose stimulation both in control cells and WSTF KD cells (Figure 3D). The protein levels of the WSTF and CHD4 remained unaltered in glucose depleted and glucose stimulated cells (Figure S2C). We suggest that an impaired B-WICH activity results in the NuRD ATPase CHD4 not being released upon glucose stimulation and thereby maintaining a closed chromatin structure at the rRNA gene promoter.

3.9 Knock down of CHD4 results in an active chromatin structure at the promoter

In order to investigate the function of CHD4 and the NuRD complex at the rRNA promoter, we used siRNA to knock down the protein and study its effect on rRNA gene transcription. Transfection with two pooled siRNAs against CHD4 resulted in a reduced protein level at 24 and 48 hours after transfection (Figure 4A). (The knock down of the individual

**FIGURE 3** The CHD4 ATPase remains on the promoter in WSTF KD cells in glucose stimulated cells. A, ChIP-qPCR analysis of SIRT1 and HDAC2 at the rRNA gene promoter in cells transfected with siRNAs against WSTF or control siRNA. Cells were glucose deprived for 18 hours (−) or glucose stimulated for six hours after 18 hours of glucose deprivation (+). The values are presented as percentages of the input signal. N = 5. mean ± SD. (SIRT1. Control siRNA *P = .044, WSTF siRNA n.s.; HDAC2. Control siRNA *P = .038, WSTF siRNA *P = .047). B, ChIP-qPCR analysis of CHD4 and histone H1 at the rRNA gene promoter in exponentially growing cells transfected with siRNAs against WSTF or control siRNA. The values are presented as percentages of the input signal. N = 3. mean ± SD. (CHD4: control siRNA vs WSTF siRNA *P = .0009). C and D, ChIP-qPCR analysis of CHD4 (C), and histone H1 (D) at the rRNA gene promoter in cells transfected with siRNAs against WSTF or control siRNA. Cells were glucose deprived for 18 hours (−) or glucose stimulated for six hours after 18 hours of glucose deprivation (+). The values are presented as percentages of the input signal. N = 4. mean ± SD. C) CHD4: control siRNA **P = .002, WSTF siRNA *P = .03; D) histone H1: control siRNA **P = .003, WSTF siRNA **P = .002)
siRNAs is shown in Figure S2D.) We next investigated the effect of the knock down of CHD4 for 48 hours on the rRNA level in response to the two different glucose concentrations. The level of 45S rRNA in glucose depleted cells remained the same as in control cells and no strong increase was observed in glucose stimulated CHD4 KD cells (Figure 4B, compare Figure 1A). This suggests that the silencing in response to glucose deprivation is impaired in the absence of CHD4. We, therefore, examined the accessibility by MNase digestion of the rRNA gene promoter in CHD4 KD cells. The chromatin configuration of the promoter was in an open state in both glucose-deprived and glucose-stimulated CHD4 KD cells (Figure 4C). This supports the findings that NuRD is involved in establishing a repressive poised chromatin state in response to glucose deprivation.

Next, we investigated the effect of CHD4 knock down on RNA pol I and UBF association with the promoter. RNA pol I associated with the promoter in both glucose deprived cells and stimulated cells, supporting the finding that 45S rRNA was expressed in both conditions (Figure 4D). The binding of UBF to the promoter was not altered by different exposures to glucose in CHD4 knock down cells (Figure 4D). We also examined the binding of c-MYC at the sites close to the promoter, and despite the fact that transcription was maintained, c-MYC did not associate with the promoter in glucose deprived CHD4 KD cell and to a low level in glucose stimulated cells (Figure 4E). This
FIGURE 4 The CHD4 ATPase is involved in silencing of the rRNA promoter in response to glucose. A. Immunoblot of whole cell extract (30 µg protein) isolated at 24 hours and 48 hours after transfection with either a single control siRNA or two combined siRNAs against CHD4. The immunoblot was probed with antibodies to CHD4, with tubulin used as a loading control. The antibodies used are marked on the right. The molecular weight marker is depicted on the left. B. qPCR analysis of rRNA gene transcription levels in cells transfected with siRNA against CHD4 or control siRNA. The control cells were grown in normal conditions; the CHD4 siRNA cells were glucose deprived for 18 hours (−glc), or glucose stimulated for six hours after 18 hours of glucose deprivation (+glc). Results are normalized to control cells under normal growth conditions. N = 4, mean ± SD. C. qPCR analysis of chromatin treated with micrococcal nuclease. A schematic diagram of the rRNA gene is drawn above the graph, where the arrow indicates the transcription start site, and primer positions are given on the x-axis (base distance from transcription start site). Control cells (full lines) and CHD4 KD cells (dashed lines) were grown in no glucose for 18 hours (−glc, black lines) or re-fed with glucose for six hours after 18 hours of glucose deprivation (+glc, red lines). The chromatin profile is presented as ±245 of undigested and MNase-digested cross-linked chromatin. N = 4, mean ± SD. D. qPCR of ChIP analysis of RNA Pol I and UBF in cells transfected with siRNA against CHD4 or control siRNA. Cells were glucose deprived for 18 hours (−) or glucose stimulated for six hours after 18 hours of glucose deprivation (+). The values are presented as percentages of the input signal. N = 5. mean ± SD. (Pol I. control siRNA **P = .005, CHD4 siRNA *P = .012; UBF. all values n.s.). E. ChIP-qPCR analysis of c-MYC in cells transfected with siRNA against WSTF or control siRNA. The region of the rRNA gene is drawn above the graph, where the arrow indicates the transcription start site, and primer positions are given for promoter (light blue) and +1kb (dark blue) regions. Cells were glucose deprived for 18 hours (−) or glucose stimulated for six hours after 18 hours of glucose deprivation (+). The values are presented as percentages of the input signal. N = 4. Mean ± SD. (control siRNA, WSTF ***P = .0005, SNF2h ***P = .00009; CHD4 siRNA. All values n.s.)

suggests that the basic level of transcription is c-MYC independent in HeLa cells, and instead is important for the increased transcription observed after 6 hours re-feeding after long-term starvation. In conclusion, CHD4 knock down results in the cells not being able to silence rRNA transcription in response to glucose deprivation, and basal transcription is maintained.

3.10 WSTF and SNF2h are recruited to the rRNA gene promoter in an opposite manner to CHD4 in response to glucose

Since WSTF KD affected the association of CHD4, we investigated the reciprocal influence; the association of B-WICH with the promoter in CHD4 KD cells. Next, we investigated the pattern by which CHD4 and B-WICH associated with the promoter in response to glucose. The B-WICH ATPase SNF2h followed the binding pattern of WSTF and displayed a reduced level at the promoter in glucose depleted cells and the level increased after glucose stimulation in control cells (Figure 4F). CHD4 knock down resulted in WSTF and SNF2h being associated with the promoter in both conditions (Figure 4F). This supports the mutual interplay between NuRD and B-WICH; CHD4 and WSTF showed opposite binding patterns at the promoter (compare Figures 3C and 4F) and they also affected each other’s binding at the promoter.

3.11 WSTF and CHD4 regulates the binding of TTF-1

CHD4 is recruited to the promoter by interacting with TTF-1 and by the promoter antisense transcript PAPAS. TTF-1 is involved in the regulation of rRNA transcription by interacting with chromatin modifying proteins, such as CHD4, TIP5 and CSB. The direct interaction between TTF-1 and chromatin factors prompted us to investigate the protein interactions between WSTF and TTF-1. No interaction between WSTF and TTF-1 could be detected in cell extracts prepared at 0.7 M NaCl (Figure 5A) or 0.42 M NaCl. RNA is also involved in the interaction, the 45S rRNA in binding to WSTF and CHD4 is recruited by PAPAS. The level of PAPAS increases when 45S rRNA transcription is reduced, and we examined the level of antisense promoter RNA produced in response to glucose and WSTF knock down. However, no increase in PAPAS in glucose depleted cells or WSTF KD cells was detected compared to glucose stimulated cells (Figure S2E).

TTF-1 also mediates loops between the promoter region and the end of the gene to activate ribosomal transcription, which led us to investigate how B-WICH or NuRD influence the association pattern of TTF-1 at the promoter. Both WSTF and CHD4 knock down changed the binding of TTF-1 to the promoter. The association of TTF-1 increased in WSTF KD cells compared to exponentially growing control cells and CHD4 knock down abolished the association of TTF-1 (Figure 5B), possibly reflecting the interaction between the proteins. This difference in binding to chromatin was not caused by differences in protein level of TTF-1 expressed, which was equal in WSTF and CHD4 KD cells (Figure S3A). To conclude, these findings suggest that direct interaction with TTF-1 alone cannot regulate the reciprocal association between B-WICH and NuRD by protein interactions.
transcriptional termination, referred to as T1-T10. To examine the function of B-WICH and NuRD in response to glucose, we investigated the binding pattern also at the Tsp, close to the spacer promoter. The association of WSTF and CHD4 with the Tsp site in response to glucose resembled that found at the promoter; the association of WSTF was low in glucose deprived cells and increased upon glucose stimulation, whereas the association of CHD4 exhibited the opposite pattern (Figure 5C, Figure S3B shows the proteins at both Tsp and the promoter in HeLa for comparison). WSTF displayed a similar pattern of association to the Tsp in HEK293 cells (Figure S1B). Although glucose stimulation resulted in a similar association pattern of WSTF and CHD4 at the two promoters, the binding of TTF-1 was different; in control cells the TTF-1 increased its binding to the Tsp in response to glucose, but remain unaltered at the promoter (Figure 5D). Similar to the exponentially growing cells, WSTF knock down resulted in a higher level of TTF-1 while knock down of CHD4 abolished to binding of TTF-1 to the promoter, in both conditions. Both WSTF and CHD4 knock down resulted in a severely reduced binding of TTF-1 to the Tsp (Figure 5D). This pattern of TTF-1 binding at the T0 site and the Tsp site indicates that CHD4 is required for binding of TTF-1, whereas the role of WSTF for the binding of TTF-1 is different. It promotes TTF-1 binding, possibly by inducing a chromatin structure favoring increased binding.
FIGURE 5  TTF-1 KD does affect the UBF level at Tsp and promoter but not rRNA transcription. A, Co-immunoprecipitation from 0.7 M NaCl cell lysates using antibodies against WSTF, TTF-1 and control IgG. The samples are marked above the lanes. The antibodies used to probe the membranes are marked on the right. The molecular weight marker is depicted on the left. B, ChIP-qPCR analysis of TTF-1 at the rRNA gene promoter in exponentially growing cells transfected with siRNAs against WSTF, siRNAs against CHD4 or control siRNA. The values are presented as percentages of the input signal. N = 3. Mean ± SD. (control siRNA vs WSTF siRNA *P = .003; control siRNA vs CHD4 siRNA *P = .002.) C, ChIP-qPCR analysis of WSTF and CHD4 at the Tsp site in cells transfected with control siRNA. Cells were glucose deprived for 18 hours (−) or glucose stimulated for six hours after 18 hours of glucose deprivation (+). The values are presented as percentages of the input signal. N = 4. Mean ± SD. (control siRNA vs WSTF siRNA **P = .0016, CHD4 +P = .017). D, ChIP-qPCR analysis of TTF-1 at the rRNA gene promoter and the Tsp site in cells transfected with siRNAs against WSTF, CHD4 or control siRNA. The region of the rRNA gene is drawn above the graph, where the arrow indicates the transcription start site, and primer positions are given for promoter (light blue) and Tsp site (green) regions. Control cells, the WSTF siRNA cells and the CHD4 siRNA cells were glucose deprived for 18 hours (−), or glucose stimulated for six hours after 18 hours of glucose deprivation (+). The values are presented as percentages of the input signal. N = 4. Mean ± SD. (Tsp: control siRNA *P = .02; promoter: control −glc vs WSTF siRNA −glc *P = .01, +glc **P = .0024; control vs CHD4 siRNA −glc. ***P = .00013, CHD4 +glc **P = .006). E, qPCR analysis of rRNA gene transcription levels in cells transfected with siRNA against TTF-1 or control siRNA. The control cells were grown in normal conditions; the TTF-1 siRNA cells were glucose deprived for 18 hours (−), or glucose stimulated for six hours after 18 hours of glucose deprivation (+). Normalized to control cells under normal growth conditions. N = 6. Mean ± SD. (TTF-1 siRNA −glc *P = .00014, TTF-1 siRNA +glc ***P = .007, TTF-1 KD −glc vs +glc **P = .00016). F, ChIP-qPCR analysis of WSTF and CHD4 at the rRNA gene promoter and the Tsp site in cells transfected with siRNA against TTF-1 and control siRNA. Cells were glucose deprived for 18 hours (−) or glucose stimulated for six hours after 18 hours of glucose deprivation (+). The values are presented as percentages of the input signal. N = 5. Mean ± SD. (Promoter: TTF-1 siRNA. WSTF **P = .002, CHD4 **P = .0005). G, qPCR of ChIP analysis of RNA Pol I and UBF at the rRNA gene promoter and Tsp site in cells transfected with siRNA against TTF-1 or control siRNA. Cells were glucose deprived for 18 hours (−) or glucose stimulated for six hours after 18 hours of glucose deprivation (+). The values are presented as percentages of the input signal. Mean ± SD. (Tsp N = 3: pol I: control *P = .04, TTF-1 siRNA *P = .03; UBF: control **P = .002, TTF-1 siRNA **P = .003, control +glc vs TTF-1 siRNA +glc **P = .005; promoter N = 5. Pol I: control siRNA **P = .002, TTF-1 siRNA 0.037; UBF: control −glc vs TTF-1 siRNA −glc *P = .017, control +glc vs TTF-1 siRNA +glc **P = .005). H, ChIP-qPCR analysis of c-MYC in cells transfected with siRNA against TTF-1 or control siRNA. The region of the rRNA gene is drawn above the graph, where the arrow indicates the transcription start site, and primer positions are given for promoter (light blue) and +1kb (dark blue) regions. Cells were glucose deprived for 18 hours (−) or glucose stimulated for six hours after 18 hours of glucose deprivation (+). The values are presented as percentages of the input signal. N = 5. mean ± SD. (Promoter. control siRNA ***P = .0007, TTF-1 siRNA *P = .02; +1kb. control siRNA ***P = .0002, TTF-1 siRNA **P = .003)

3.12  TTF-1 affects UBF levels without affecting the 47S rRNA levels

Since both WSTF and CHD4 KD altered the association pattern of TTF-1 at the promoter and Tsp, we investigated the effect of TTF-1 on the ribosomal transcription in response to glucose. TTF-1 knock down was performed using two siRNAs (Figure S3C). Knock down of TTF-1 did not alter the transcription of rRNA in response to glucose; glucose deprivation reduced the rRNA level and glucose stimulation for 6 hours increased the level above the level in exponentially growing control cells, similar to the effect observed in control cells except for a reduction in glucose deprived cells (Figure 5E). TTF-1 knock down did not change the association of WSTF or CHD4 with the promoter in glucose deprived or stimulated cells, and the pattern resembled that observed in control cells (Figure 5F). However, the association of WSTF and CHD4 was abolished at the Tsp site in both glucose deprived and stimulation cells, indicating that the association of factors at Tsp is more sensitive to environmental changes when TTF-1 level is low.

We also investigated the binding of RNA pol I and UBF in TTF-1 knock down cells. The increased rRNA transcription in glucose stimulated TTF-1 KD cells was reflected in a higher RNA pol I binding to the promoter and the Tsp (Figure 5G). The UBF level at the Tsp in TTF-1 KD cells responded to the glucose level, however, weaker at the Tsp than in control cell (Figure 5G). In contrast, the UBF level did not change in response to glucose at the promoter, but the association was higher in TTF-1 KD cells (Figure 5G). The UBF level at the Tsp in TTF-1 KD cells responded to the glucose level, however, weaker at the Tsp than in control cell (Figure 5G). A slightly higher UBF level at the promoter in TTF-1 KD cells was also observed in exponentially growing cells (Figure S3D). The higher level of UBF at the promoter in TTF-1 KD cells had no effect on the recruitment of c-MYC to the promoter or the site 1 kb downstream of TSS (Figure 5H). We conclude that the increased transcription upon stimulation of glucose does not depend on the presence of TTF-1 at the promoter or Tsp, although TTF-1 reorganized the composition of UBF at the site. However, a minor effect could be detected on the silencing of rRNA, which could be a result of the higher UBF levels. Taken together, these results indicate that the B-WICH and the NuRD complexes form a regulatory reciprocal pair which is involved in the shift in chromatin configurations at the rRNA gene promoters in response to environmental cues. The TTF-1 keep the chromatin structure in balance and the shift allows for proper recruitment of regulatory factors (Figure 6).
4 | DISCUSSION

We here show that the B-WICH complex activates RNA Pol I transcription in response to nutrients by reverting a silent, permissive state imposed by the NuRD chromatin remodeling complex. The B-WICH complex is involved in the activation of transcription by acting at an early step to remodel the chromatin at the rRNA gene promoter into an open, active state. Activation of RNA pol I transcription requires several steps and differs between activation after short-term inactivation and long-term silencing. Short-term regulation of ribosomal transcription affects the elongation rate of transcription, whereas de novo initiation occurs after long-term changes, such as cell cycle arrest at G0/G1, and relies on modification of the local chromatin structure, transcription factor binding and assembly of the RNA pol I preinitiation complex. The effect of WSTF knock down resembles a state that requires de novo initiation, in which full assembly of the preinitiating complex is needed to resume transcription. The NuRD complex establishes a poised silent chromatin state at the promoter by remodeling a nucleosome over the start site to exclude transcription factors and the general RNA pol I machinery. Based on our data, we propose that B-WICH counteracts this repressive, poised chromatin state at the promoter by remodeling a nucleosome over the start site to exclude transcription factors and the general RNA pol I machinery to bind to promoter.

Several different silencing mechanisms are operating on the rRNA genes involving different chromatin factors and chromatin remodeling complexes, such as NML-SIRT1 eNoSC, the histone H1 and HP1, the CHD4-NuRD complex, and the TIP5-SNF2h NoRC complex. The eNoSC complex is involved in energy-sensing and establishes a silent chromatin state by inducing deacetylations and silent methylations at the promoter in response to the low redox state present in glucose starved cells. However, the component SIRT1 did not associate with the promoter in glucose depleted WSTF KD cells, indicating that the inhibition of rRNA transcription in B-WICH impaired cells does not involve deacetylation by the eNoSC complex. It has been reported that NML, the defining subunit in the eNoSC complex, binds to the 45S rRNA and associates with and silences the promoter upon release from RNA. This in turn suggests that eNoSC is not involved in the induction of RNA gene silencing at energy depletion, but rather acts by maintaining a silenced state at the promoter during prolonged low energy levels. Energy depletion is also regulated by phosphorylation of the RNA Pol I machinery; several kinases control the association of RRN3/TIF1-A and SL1/TIF-1B with RNA Pol I at the promoter. A low energy level is sensed by the AMPK and it introduces an inhibitory phosphorylation on RRN3/TIF1-A, which in turn blocks the binding to RNA pol I. Nevertheless, RRN3/TIF1-A was excluded from the promoter in cells with an impaired B-WICH, which supports that it acts by changing the chromatin configuration.

Histone H1 and the NuRD also associated with the RNA pol I promoter in glucose deprived cells and were released upon glucose stimulation, connecting these factors to gene repression in response to the energy state of the cells. We link the B-WICH to the activation of the poised state established by NuRD, since the CHD4 specifically accumulated in WSTF KD cells. CHD4 is a peripheral subunit in the NuRD complex and easily released from the HDACs which explains the release of HDAC2 in WSTF KD cells. Furthermore, the NuRD keeps the promoter in an unmethylated state, establishing instead a permissive poised state with bivalent histone marks, H3K4me3 and H3K27me3, on the nucleosome at the promoter. B-WICH does not act on DNA-methylated promoters in HeLa cells, instead, we propose that it counteracts
the CHD4 activity in the NuRD complex, possibly by rearranging nucleosome positioning at the promoter.

Higher order chromatin structures of rDNA, which are formed by chromatin loops that also interact with the nuclear matrix, regulate the transcriptional output. In particular, TTF-1 and c-MYC are associated with the formation of higher order chromatin to give full transcriptional activity. TTF-1 has many functions in rRNA gene transcription apart from interacting with the transcription termination site of the 45S gene; CHD4, CSB, and TIP5 in the NoRC complex interact with TTF-1 and regulate silencing or activation of transcription.20,34 TTF-1 in turn is regulated by its localization, and upon starvation it leaves the nucleolus in an ARF and nucleophosmin 1 dependent manner.57,58 Our results show that TTF-1 loading at the promoter was not dependent on the glucose level, whereas that at Tsp in the spacer promoter was. However, knock down of TTF-1 suggests that the association of factors at Tsp did not affect the transcription of rRNA gene transcription, but the binding pattern of UBF. This suggests that TTF-1 affects the underlying chromatin structure, particularly at the promoter. The higher loading of UBF upon TTF-1 knock down did not affect activation of transcription or the association of B-WICH and NuRD, but our results indicate that silencing is slightly impaired. We suggest that the promoter is the most important element for transcription in human cells and that TTF-1 is involved in maintaining a proper chromatin structure. One role that NuRD and B-WICH play at the promoter is to keep the binding of TTF-1 in balance.

c-MYC is also involved in rRNA gene transcription by forming chromatin loops which brings the upstream region and the downstream region of the 45S gene together and attaches the IGS to the nuclear matrix structures in rat and mouse cells.11,12 In human cells, only c-MYC binding sites (E-boxes) in the intergenic spacer, including the promoter, loop and attach to matrix structures in serum activated cells.12 We only investigated the E-boxes in the proximity of the promoter in this study, and we show that c-MYC was bound to the promoter site upon glucose refeeding, indicating that higher order structures were formed. The binding of c-MYC binding required an open chromatin configuration, suggesting a multifactorial mechanism in the transition from an inactive permissive poised state to full transcriptional response. First the promoter chromatin is altered to allow for transcription factors to bind and induce basal transcription. To reach full transcription, TTF-1 or c-MYC must associate and probably form chromatin loops. The B-WICH and NuRD are mainly responsible to alter the chromatin configuration as a first step, but they are also involved in stabilizing TTF-1 and allowing for c-MYC to bind.

Ribosomal transcription is tightly regulated during development (for review 2). The WSTF protein is ubiquitously expressed in adult tissues,59 but during development the protein is strongly expressed in the early closing neural tube and later in specific neural structures, such as the neural tube and the migrating neural crest.60 WSTF is haploinsufficiently expressed in William syndrome patients, and is closely correlated to craniofacial development and a number of other developmental abnormalities found in these patients. Our finding that B-WICH releases the CHD4 ATPase and establishes an active permissive chromatin state (Figure 6) indicates that cells with low levels of WSTF have an impaired response to external stimuli which may lead to defects in development and differentiation.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

A. Rolicka, Y. Guo, M. Arsenian-Henriksson, A.-K. Östlund Farrants designed research; A. Rolicka, Y. Guo, A. Ganez Zapater, K. Tariq, J. Quin, A. Vintermist, F. Sadeghifar, A.-K. Östlund Farrants performed and analyzed data; A. Rolicka, Y. Guo, A.-K. Östlund Farrants wrote the manuscript; A. Ganez Zapater, K. Tariq, J. Quin, A. Vintermist, F. Sadeghifar, M. Arsenian-Henriksson edited the manuscript.

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SUPPORTING INFORMATION
Additional Supporting Information may be found online in the Supporting Information section.

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