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Nucleolar Dysfunction in Huntington's Disease

Junghee Lee[1,2,#], **Yu Jin Hwang**[3,#], **Hyun Ryu**[2], **Neil W. Kowall**[1,2], and **Hoon Ryu**[1,2,3,*]
[1]VA Boston Healthcare System, Boston, MA 02130

[2]Boston University Alzheimer's Disease Center and Department of Neurology, Boston University School of Medicine, Boston, MA 02118

[3]WCU Neurocytomics Group, Department of Biomedical Sciences, Seoul National University Graduate School, Seoul 110-799, South Korea

Abstract

Huntington's disease (HD) is a fatal genetic disorder characterized by triad clinical symptoms of chorea, emotional distress, and cognitive decline. Genetic mutation in HD is identified by an expansion of CAG repeats coding for glutamine (Q) in exon 1 of the *huntingtin* (*htt*) gene. The exact mechanism on how mutant htt leads to the selective loss of medium spiny neurons (MSNs) in the striatum is still unknown. Recent studies suggest that nucleolar stress and dysfunction is linked to the pathogenesis of HD. Alterations of the nucleolar activity and integrity contributes to deregulation of ribosomal DNA (rDNA) transcription in HD pathogenesis. Furthermore, epigenetic modifications in the nucleolus are associated with neuronal damage in HD. In this review, we discuss about how post-translational modifications of upstream binding factor (UBF) are affected by histone acetyltransferase and histone methyltransferase and involved in the transcriptional regulation of rDNA in HD. The understanding of epigenetic modulation of UBF-dependent rDNA transcription in the nucleolus may lead to the identification of novel pathological markers and new therapeutic targets to treat HD.

Keywords

Huntington's disease; nucleolus; upstream binding factor (UBF); rDNA transcription; acetylation and methylation; epigenetics

1. INTRODUCTION

1-1. From heritable dancing disorder (chorea) to Huntington's disease (HD)

Huntington's disease (HD) is an autosomal dominant genetic disorder featured by neurological symptoms with chorea (uncoordinated movement), psychiatric symptoms, and memory loss. The frequency of HD is reported as high as 10 cases per 100,000 and having a new mutation rate of 1–3% [1]. The occurrence of HD is not limited to a specific region or population but found throughout the globe in all races and ethnic groups [2]. The shores of

*Corresponding author: Hoon Ryu, Ph.D., VA Boston Healthcare System, Building 1A, Rm 109, 150 S Huntington Ave., Boston, MA 02130, Tel: 1-857-364-5910; Fax: 1-857-364-4540, hoonryu@bu.edu.
#Equally contributed.

Lake Maracaibo in Venezuela particularly have the highest HD frequency. The number of HD patients in United States alone reach 30,000 and those at genetic risk of HD is considered to be 200,000.

Before 17th century, Huntington's disease (HD) was a mysterious dancing disorder (chorea) that shows jerking and twitching of muscles. Due to the lack of medical knowledge at that time, the people with involuntary movement as a feature of HD were being regarded as witches possessed by the devil [3]. In 1872, George Huntington, an American physician, provided the first clinical description of HD and its hereditary nature [4]. The initial understanding of HD as a chronic encephalitis has been replaced by Jergelsma in 1908, who described the characteristic neuropathological alterations affecting the basal ganglia that are now established as the imperative pathoanatomical feature of HD [5,6]. Gross atrophy of the neostriatal nuclei, the caudate nucleus, and putamen, combined by detectable neuronal loss and astrogliosis are the most salient neuropathological alterations of HD [7–9]. Within HD, not all striatal neurons are equally influenced, and there appears to be a selective pattern of neuronal vulnerability and topographic susceptibility. During the earliest stage of disease medium-sized spiny neurons are most severely affected while intrinsic locally arborizing aspiny striatal interneurons are comparably spared [9–15].

Most importantly, the Huntington's disease Collaborative Research Group (HCRG) presented in 1993 that a previously unidentified 'interesting transcript (IT) 15′ on human chromosome 4 was mutated in patients with HD and genetically linked to HD [16]. HCRG's reported HD-related lethal gene was referred to as huntingtin (htt). The expansion of the wildtype htt allele that typically contains 15–35 CAG triplets in exon 1 to 36 or more repeats has been determined to be the mutation of htt gene. Furthermore, HD is considered to be related to other neurodegenerative diseases such as spinal and bulbar muscular atrophy (SBMA, also referred to as Kennedy's disease (KD)) and spinocerebellar ataxias (SCAs) which are also caused by similar trinucleotide CAG repeat mutations. To be more specific, SBMA occurs when androgen receptor (AR) gene is mutated while the expansion of a polyglutamine tract within the SCA1 gene product ataxin-1 causes SCA1 [17, 18]. The significance of CAG expansion is observed in cases where affected individuals with greater number of CAG repeats show younger age of onset. In addition, there is a strong inverse relationship between age of onset and CAG repeat number in HD.

The cytoplasmic and vesicular protein, htt, is ubiquitously expressed and heterogeneously found in neurons throughout the brain. Numerous hypothetical pathologic mechanisms have been proposed since discovering *htt* gene but a direct pathway from the genetic mutation to neuronal degeneration has yet to be established. Even though the definitive functionality of htt remains unknown, it has been postulated that htt is involved in intracellular transport, autophagy, transcription, mitochondrial function, and signal transduction. On the other hand, mutant htt (mthtt) results in the inhibition of fast axonal transport and destabilization of microtubules within the cell [19, 20]. Within heterozygous HD, both normal and mutant alleles are expressed. As a "gain of function", the expression of mutant htt protein is toxic and it triggers the pathologic cascades of HD. In contrast, the deletion of the normal htt gene is also lethal indicating that the function of normal htt is essential in survival [21–28]. The engagement in pathologic protein-protein interactions between mutant htt and its proteolytic fragments is well established and accepted. This engagement leads to alterations of cellular pathways that make neurons to be more vulnerable to generic stresses, ultimately resulting in neuronal damage and death [29]. Additionally, mutant htt interactomes involve transcriptional dysregulation, mitochondrial dysfunction, proapoptotic signaling, oxidative injury, excitotoxicity, inflammatory reactions, and malfunctioning proteolysis. Since the first clinical description of HD by George Huntington, there has been a strong progression

towards the understanding of the disease mechanisms. However, a treatment to prevent the onset or to delay the insidious and unyielding course of HD is currently unavailable [30].

Epigenetic alterations in HD—A term “epigenetics” was described by Dr. Waddington to explain the biological events that are unexplainable by conventional genetic principals [31]. The field of epigenetics has evolved since its introduction, and is now defined as the study connecting genotype to phenotype in the absence of DNA sequence alteration [32]. In consideration to this, epigenetics is regarded as a highly beneficial field to investigate features and mechanisms responsible for the temporal and spatial control of gene activity regulated by processes beyond mutations in DNA sequence [33]. Epigenetic modifications explain for and encompass an array of molecular modifications to both chromatin and DNA, including ncRNAs regulation. For many genes in general, they contain DNA methylation sites (CpG islands) in their promoters [34]. This indicates that marked hypo- or hyper-DNA methylation may explain for significant components of the molecular and pathogenic complexity of human genomes. Expanding number of evidence suggests and point towards the alterations of epigenetic modifications to constitute a basic molecular mechanism contributing to HD pathogenesis. What the understanding of epigenetic mechanisms hold for is the opportunity to gain imperative insights leading to the identification of novel biological markers and therapeutic interventions to treat HD [35].

A growing body of evidence indicates that nucleolar stress and dysfunction is linked to the pathogenesis of HD. The nucleolus is a subnuclear compartment possessing the transcription machinery of ribosomal genes and the ribosomal DNA (rDNA) encoding ribosomal RNA (rRNA) [36]. The rDNA is organized as tandem repeats in the nucleolus and is transcribed into 47S precursor rRNA by a nucleolar transcription complex, consisting of RNA polymerase I and other co-regulatory factors [37]. Neurons have prominent nucleoli but the role of this structure and the regulatory mechanism of rDNA transcription are poorly understood [38]. RNA polymerase I and upstream binding factor (UBF) are major molecular components for the format ion of active nucleolar organizer regions and maintenance of rRNA transcriptional activity [39]. It has previously known that nucleolar accessory bodies (Cajal bodies) are associated with disorders caused by expansions of CAG repeats within genes, including HD [39]. Recently, our group and others have found that alteration of epigenetic components and deregulation of transcriptional machinery are directly involved in the down regulation of ribosomal RNA (rRNA) expression and neuronal damage in HD [40, 41] Together, in the present paper, we are going to address and discuss the epigenetic changes and mechanism that are associated with the nucleolar-dependent pathogenesis in HD.

2. Epigenetic Modifications in the nucleolus of HD

2-1. UBF acetylation in HD

UBF is a nucleolar transcription factor of the high mobility group (HMG) protein family and contains six HMG box DNA binding motifs. UBF consists of two polypeptides (UBF1 and UBF2, 97 and 94 kDa, respectively), which arise from alternative splicing of a single transcript [42]. UBF1 and 2 form hetero- and homodimers but UBF2 is five-fold less active than UBF1, and mechanisms by which UBF2 provides such poor transcriptional activity are not known [43]. UBF is essential for rDNA transcription by inducing remodeling of ribosomal gene chromatin and further playing a structural role by binding to other sequences across the entire rDNA [36]. UBF targeting to regions of heterochromatin is sufficient to induce large-scale chromatin decondensation [44]. UBF binding throughout the rDNA gene repeat might therefore contribute to the formation of the active chromatin state of rDNA genes [45]. Recently, we found that transcriptional modulation of rDNA is altered in HD, and both UBF and CREB binding protein (CBP) contribute to altered nucleolar chromatin

remodeling and rRNA expression in HD. We found that protein levels UBF1, CBP, and UBF-mediated transcriptional activity of rDNA are impaired in cellular and animal model of HD as well as in HD patients [40]. Our study shows that UBF1 protein levels in the striatum were significantly decreased and the 45S was down re gulated in R6/2 mice in comparison with WT controls. These data indicate that abnormal protein level of UBF1 protein levels correlate with impaired ribosomal transcription in HD mice.

Post-translational modifications of UBF, such as acetylation and phosphorylation, have an important role in the control of rDNA transcription [46]. Transcription factor acetylation is responsible for both activation and subnuclear localization [47]. CBP functions as a HAT in acetylating histones that contributes to transcription by remodeling the chromatin structure and functions as a transcriptional cofactor [48]. A loss of CBP function interferes with transcription by altering the acetylation level of histones and chromatin structure in neurons and also by inhibiting recruitment of the basal transcription machinery to the promoter [49, 50]. Interestingly, the sequestration of CBP molecule by mthtt protein was found in intranuclear inclusions of striatal neurons and other neurons of HD [22, 51] (Figure 1). The polyQ repeats in mthtt interact physically with CBP and deregulate its intrinsic HAT activity and transcriptional coactivator function [52, 53]. Accordingly, the blocking of CBP activity by mthtt causes the hypoacetylation and hypermethylation of histone proteins and the subsequent transcriptional dysfunction of neurons in HD [54–58]. These specific interactions and epigenetic modifications are attributable to pathological transcriptional dysfunction [59]. Importantly, we determined that CBP interacts with UBF1, acetylates UBF1, and modulates rDNA transcription in HD. UBF acetylation is also important for cell cycle-dependent regulation of rRNA expression. It has been proposed that the transcription of rDNA is regulated upon the influence of two opposite processes through UBF acetylation by CBP and deacetylation by HDAC [26]. In this context, CBP-dependent acetylation of UBF provides a thread of mechanism on the transcription activation of rDNA [46]. Indeed, we found that the acetylation levels of UBF1 are significantly lowered due to deregulation of CBP function in both HD cells and HD mice. Our data shows that UBF1 and CBP interact in intact neurons. Reduced levels of acetylated UBF1 correlate with the reduced CBP level and activity in HD. We further demonstrated that the HAT activity of CBP directly controls UBF1 acetylation and this was found using a HAT do main deletion mutant of CBP that caused a marked reduction of UBF1 acetylation. Our findings indicate that the neurodegenerative process in HD is associated with changes in adaptive rDNA transcription caused by altered CBP acetyltransferase that affects the balance of acetylated and deacetylated UBF1 [40, 22] (Figure 1).

The LC-MALDI-MS/MS analysis identified the specific acetylation site of UBF1 from *in vitro*-acetylated UBF protein by CBP [40]. CBP acetylates UBF1 at K352 that resides in the H MG3 domain. Mutations at K352 to alanine (A), glutamine (Q), and arginine (R) abrogates the acetylation of UBF1, as well as the transcriptional activation of UBF1 in response to CBP. This data confirms that the acetylation of UBF1 by CBP has a pivotal role in rDNA gene transcription. Consequently, due to the lack of CBP-dependent acetylation of UBF1, UBF1-induced rDNA expression is disrupted in HD models [46, 60]. It is unclear, however, whether mtHtt and HD-related cellular changes modulate the association of HDAC to the UBF1 complex. Future study remains to define precisely how UBF1 protein is deacetylated and what other factors are involved in this process. This will provide a better understanding of the rDNA transcription and ribosomal synthesis in the pathogenesis of HD.

2-2. UBF methylation in HD

The motor symptoms of HD are largely a consequence of profound neuronal damage in the GABAergic MSNs [3]. Deregulation of chromatin remodeling is regarded as one of the

mechanisms by which mutant mthtt contributes to cell death of MSNs in the striatum [3]. Mthtt not only blocks the intrinsic HAT activity of CBP but also induces epigenetic enzymes such as histone methyltransferases (HMTs) [52, 61]. These specific epigenetic alterations show how mthtt modulates transcriptional signaling cascades that initiate a number of downstream pathophysiological mechanisms relevant to HD. ERG-associated protein with SET domain (ESET/SETDB1) is a histone H3K9-specific methyltransferase that contains both tudor and methyl-CpG binding domains that converge transcription and RNA processing factors [62]. It also acts as a signature motif for proteins regulating methylated DNA silencing [62]. SETDB1 is involved in neuronal dysfunction through its histone methyltransferase activity and the epigenetic silencing of neuronal genes [56]. However, the roles of SETDB1 on nucleolar chromatin landscaping and rDNA transcription have not been fully investigated yet [63]. We have recently found that elevations of *SETDB1* expression and H3K9me3 level are correlated with transcriptional deregulation and neurodegeneration in HD [56, 64]. To examine the links between SETDB1 activity and nucleolar function, we evaluated whether SETDB1 interacts with UBF and contribute to the chromatin organization of the nucleolus and rDNA transcription in HD. First, we found that increased levels of methylated UBF are correlated with the increased SETDB1 activity, a pathological event found in HD (Figure 2). As we expected, the levels of SETDB1 protein and methylated UBF were significantly increased in the striatum of R6/2 transgenic HD mice and human HD patients (unpublished data). Second, we determined that SETDB1 physically interacts with UBF and methylates it in intact neurons. The methyltransferase activity of SETDB1 is directly responsible for UBF methylation. Accordingly, SET domain deletion mutants of SETDB1 resulted in a marked reduction of UBF methylation. In general, K (lysine) residues are mono-, di, or trimethylated, and the status of methylation contributes to different functional outcomes [65]. We confirmed that UBF is presented as a trimethylated form in intact cells. In addition, UBF is trimethylated by SETDB1 *in vitro*. We performed LC-MALDI-MS/MS analysis *in vitro* on UB-MG protein methylated by SETDB1 and identified the specific methylation site of UBF. We found that SETDB1 methylates UBF at the K232/254 residues of the HMG2 domain (unpublished data). Accordingly, mutations at K232/254 blocked UBF trimethylation and derepressed the transcriptional suppression of rDNA by methylated UBF in response to SETDB1. Furthermore, methylation site mutant UBF (K232/254A) restored the transcriptional level of intermediate (5′-ETS) and mature (18S and 28S) rRNA levels while SETDB1 decreased the expression of rRNA through the methylation of wild type UBF. We also determined that 45S levels were down regulated by SETDB1. Our data suggests that the increased level of methylated UBF is correlated with deregulation of ribosomal transcription in HD, and the SETDB1-dependent trimethylation of UBF plays a direct role in the regulation of nucleolar chromatin plasticity (Figure 2). Targeting of UBF trimethylation to regions of heterochromatin is sufficient to induce large-scale chromatin condensation in the nucleolus. In this paradigm, the binding of trimethylated UBF throughout the rDNA gene repeat might contribute to the formation of the inactive chromatin state of rDNA genes. It remains to be determined, however, how mthtt and HD-related cellular changes modulate the association of ESET to the UBF complex. It will also be important to precisely define the demethylation mechanism of UBF in order to more fully understand the dynamic role of UBF on nucleolar chromatin remodeling and rDNA transcription in HD.

3. Therapeutic approaches for improving the nucleolar function in HD

Genetic mutations are irreversible but epigenetic modifications are reversible processes. In this context, from a therapeutic perspective, epigenetic modifications are regarded as a useful therapeutic target. Small compounds that dynamically inhibit HDAC activity can modulate the status of rDNA transcription by post-translational modifications of histone and non-histone molecules, as well as remodeling of the chromatin structure in HD. Therefore,

the development of such drug agents that realign the epigenetic balance and subsequently improve HD-related deficits in the nucleolus is necessary in HD-related researches. Because the acetylation on lysine residue in the UBF transforms nucleolar chromatin structure locally and results in increased DNA transcription, HDAC inhibitors can promote acetylation of UBF and transcription of rDNA. Indeed, trichostatin A, a deacetylase inhibitor, enhances UBF acetylation and rDNA gene expression [39, 26]. We also found that sodium butyrate elevates rDNA transcription in mutant HD Q111 cells and WT Q7 cells in the presence of UBF1 in a dose-dependent manner [40]. Our group has shown that the increased acetylation of transcription factors by three structurally distinct HDAC inhibitors improves neuronal survival in response to oxidative stress [66]. Collectively, these studies are consistent with a model in which altered rDNA transcription is an additional mechanism contributing to the protective effects of HDAC inhibitors in neurons. HDAC inhibitors have been preclinically tested in many neurodegenerative conditions, including animal models of HD, amyotrophic lateral sclerosis (ALS), and multiple sclerosis [49, 54–56, 67, 68]. Taken together, it is encouraging that HDAC inhibitors may improve phenotypes by upregulating rDNA transcription that is repressed in HD [40, 49, 54–56]. However, the underlying mechanisms whereby HDAC inhibitors modulate the function of nucleolus remain to be investigated. Thus, further studies will be needed to clarify the contribution of UBF1 acetylation and rDNA transcription to the neuroprotective effects of HDAC inhibitors.

4. Conclusions

Alterations of epigenetic modification in the nucleolus are closely associated with HD pathogenesis. In this context, posttranslational modifications of UBF play an important role in the regulation of nucleolar chromatin structure and transcription in striatal cells. Interestingly, an imbalance of transcriptional homeostasis through UBF hypoacethylation and hypermethylation provides another layer of pathological mechanisms that are relevant to HD. While the increased acetylation of UBF by CBP up regulates the transcription of rDNA under normal condition, the decreased acetylation of UBF due to the deficiency of CBP HAT activity le ads to the down regulation of rDNA transcription in HD. Since HDAC inhibitor increases the acetylation of UBF, the use of small compounds targeting HDAC in particular may be a successful therapeutic strategy for ameliorating the rDNA trascription in HD [40]. However, approaches using HDAC inhibitor-based therapeutic interventions need improved target specificity for UBF-dependent nucleolar transcription in HD. Otherwise, the trimethylation of UBF by HMT elevates the occupancy of UBF to the promoter region of rDNA, which condensates the structure of nucleolar chromatin, and leads to the down regulation of rDNA transcription. Prevention of UBP hypermethylation by inhibiting HMT activity may also be useful therapeutic targets for treating HD. It is evident that the status of UBF acetylation and methylation is an important marker directly or indirectly associated with transcriptional abnormality in the nucleolus of HD [40]. However, it remains to be further investigated whether epigenetic alterations are a fundamental aspect of HD pathogenesis or not. Accordingly, future studies are required to address whether epigenetic alterations in the nuclelous play a key role as a maker of disease progress in HD.

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Abbreviations

HD Huntington’s disease

htt	huntingtin
mthtt	mutant htt
MSNs	medium spiny neurons
rDNA	ribosomal DNA
rRNA	ribosomal RNA
UBF	upstream binding factor
HAT	histone acetyltransferase
Pol I	RNA polymerase I
HMT	histone methyltransferase
CBP	CREB binding protein
HMG	high mobility group
ESET	ERG-associated protein with SET domain
HDAC	histone deacetylase

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Highlights

- CBP is localized into the nucleus and acetylates UBF.
- The decreased level of UBF acetylation is correlated with the reduction of rDNA transcription in HD.
- Trimethyation of UBF leads to nucleolar chromatin condensation and impairs rDNA transcription in HD
- Epigenetic modifications of UBF contribute to the nucleolus-dependent pathogenesis of HD.

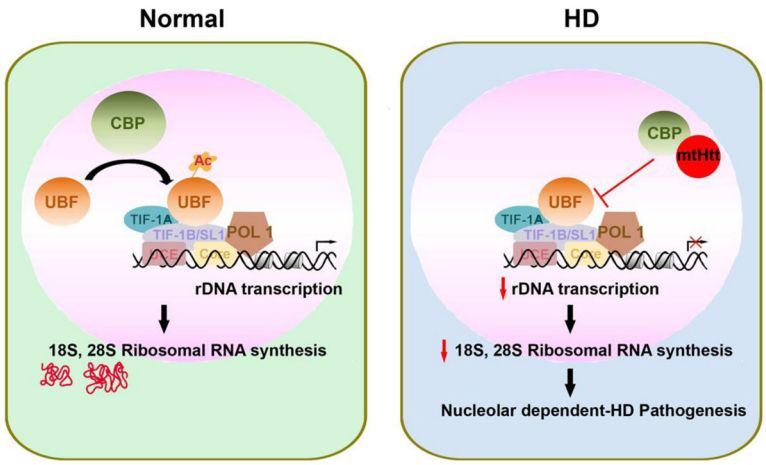

Figure 1. Mutant huntingtin (mthtt) sequestrates CBP and disrupts CBP-mediated UBF activation in the nucleolus of HD

In normal conditions, CBP maintains the acetylation status of UBF through HAT activity and regulates the transcription of rDNA by interacting with transcriptional complexes including RNA polymerase I (Pol I). In HD, mthtt sequestrates CBP in nuclear inclusions (aggregate formation) and deregulates UBF-dependent nucleolar transcription. Consequently, imbalanced rDNA transcription leads to neuronal damage in HD.

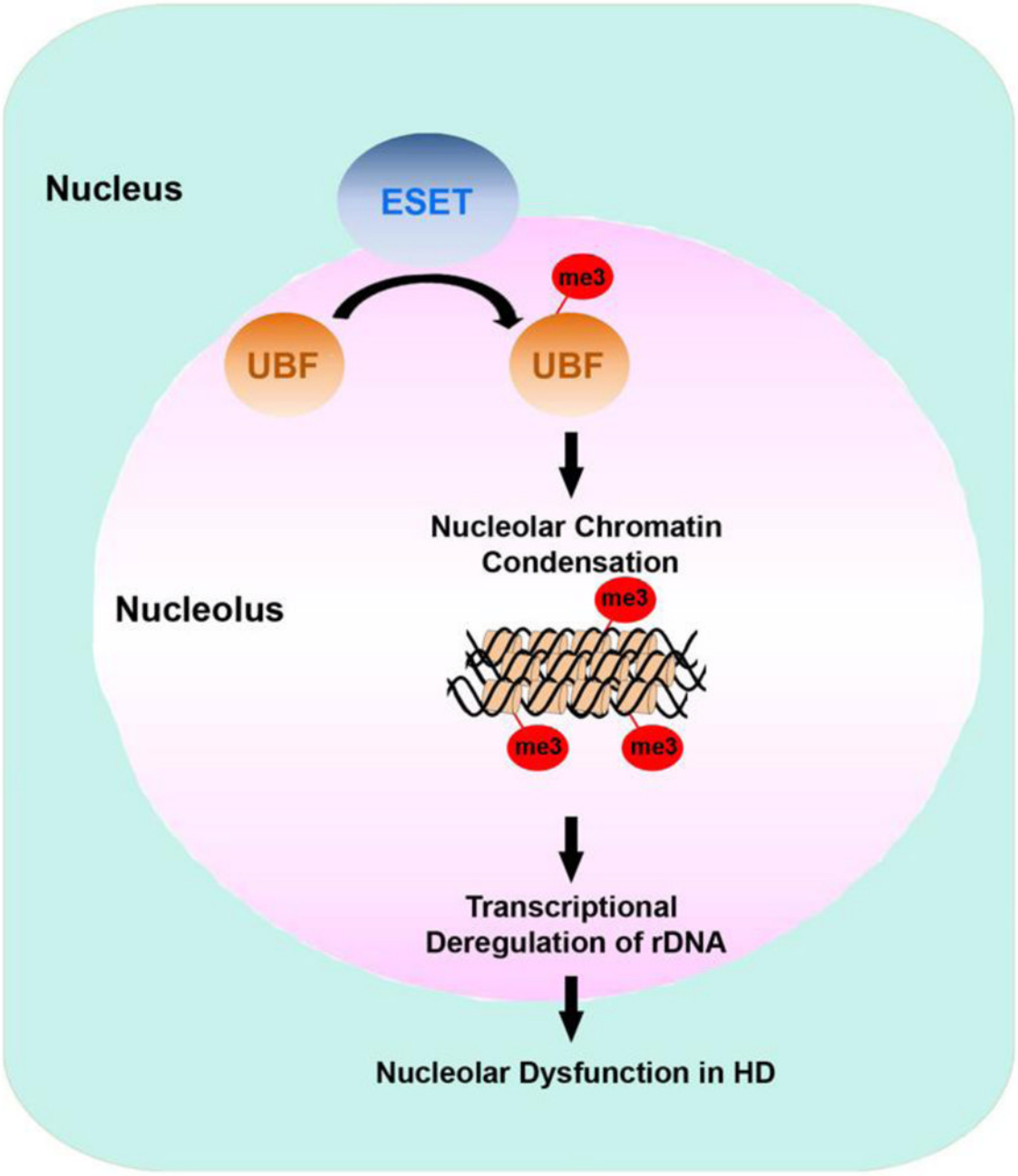

Figure 2. A scheme represents an epigenetic mechanism that abnormal activity of histone H3K9-specific methyltransferase (ESET/SETDB1) leads to UBF trimethylation and impaired rDNA transcription in HD

ESET methylates UBF and UBFme3-dependent nucleolar chromatin condensation results in the repression of rDNA transcription, and subsequent reduction of 18S and 28S RNA. Down regulation of rDNA transcription contributes to striatal dysfunction and neurodegeneration in HD.