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## Trichostatin A Relieves Growth Suppression and Restores Histone Acetylation at Specific Sites in a FUS ALS/FTD Yeast Model

Seth A. Bennett<sup>§,£</sup>, Samantha N. Cobos<sup>§,#</sup>, Melagras Mirzakandova<sup>§</sup>, Michel Fallah<sup>§</sup>, Elizaveta Son<sup>§</sup>, George Angelakakis<sup>§</sup>, Navin Rana<sup>§</sup>, Muna Hugais<sup>§</sup>, Mariana P. Torrente<sup>§,£,#,\*</sup>

<sup>§</sup>Department of Chemistry, Brooklyn College, Brooklyn, New York, 11210, United States

<sup>£</sup>Ph.D. program in Biochemistry, The Graduate Center of the City University of New York, New York, New York, 10016, United States

<sup>#</sup>Ph.D. program in Chemistry, The Graduate Center of the City University of New York, New York, New York, 10016, United States

### Abstract

Amyotrophic Lateral Sclerosis (ALS) is an incurable neurodegenerative disease which often occurs concurrently with frontotemporal dementia (FTD), another disorder involving progressive neuronal loss. ALS and FTD form a neurodegenerative continuum and share pathological and genetic features. Mutations in a multitude of genes have been linked to ALS/FTD, including *FUS*. The *FUS* protein aggregates and forms inclusions within affected neurons. However, the precise mechanisms connecting protein aggregation to neurotoxicity remain under intense investigation. Recent evidence points to the contribution of epigenetics to ALS/FTD. A main epigenetic mechanism involves the post-translational modification (PTM) of histone proteins. We have previously characterized the histone PTM landscape in a *FUS* ALS/FTD yeast model finding decreased acetylation on lysine residues 14 and 56 of Histone H3. Here, we describe the first report of amelioration of disease phenotypes by controlling histone acetylation on specific modification sites. We show that inhibiting histone deacetylases (HDACs), via treatment with Trichostatin A (TSA), suppresses the toxicity associated with *FUS* overexpression in yeast by preserving the levels of H3K56ac and H3K14ac without affecting *FUS* expression or its aggregation. Our data raises the novel hypothesis that the toxic effect of protein aggregation in neurodegeneration is related to its association with altered histone marks.

Altogether, we demonstrate the ability to counter the repercussions of protein aggregation on cell survival by preventing specific histone modification changes. Our findings launch off a novel

\*Corresponding Author: Mariana Torrente – mariana.torrente@brooklyn.cuny.edu.

Author Contributions

Conceived and Designed the Experiments: S.A.B., M.P.T.

Performed the Experiments: S.A.B., S.N.C., M.M., M.F., E.S., G.A., N.R., M.H

Analyzed the Data: S.A.B., M.P.T.

Wrote the Paper: S.A.B., S.N.C., M.P.T.

ASSOCIATED CONTENT

Supporting Information

Supplementary materials outlining detailed Materials and Methods information, Supplementary Figures presenting Trichostatin A's effect on *FUS* expression and aggregation, its effect on TDP-43 proteinopathy models and its impact on other histone modification levels, as well as Supplementary References are available free of charge. (PDF)

mechanistic framework that will enable alternative therapeutic approaches for ALS/FTD and other neurodegenerative diseases.

## Keywords

FUS; Amyotrophic Lateral Sclerosis; Frontotemporal Dementia; Histone Acetylation; Trichostatin A

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Amyotrophic Lateral Sclerosis (ALS) is a relentless, fatal neurodegenerative disease characterized by the loss of motor neurons in the spinal cord, brain, and brainstem leading to progressive paralysis.<sup>(1)</sup> ALS shares genetic and pathological features with frontotemporal dementia (FTD). FTD primarily affects the frontal and temporal lobes of the brain causing deficits in the ability to reason and control movement.<sup>(2)</sup> ALS and FTD lie on two ends of a disease continuum.<sup>(2)</sup> Prognosis for ALS/FTD patients is poor. There is no cure, and no therapy is able to stop or modify disease progression.

Mutations in a large number of genes have been associated with ALS/FTD. The protein products of these mutant genes aggregate and form inclusions within affected neurons, leading to their demise. Among these, Superoxide Dismutase 1 (SOD1), TAR DNA-binding protein 43 (TDP-43) and Fused in Sarcoma (FUS) have been thoroughly studied. FUS is a RNA-binding protein involved in various processes, including splicing and DNA damage repair.<sup>(3)</sup> Despite intense investigation, the molecular channels connecting protein aggregation to neurotoxicity remain elusive.

Epigenetics refers to heritable alterations in gene expression occurring without modification to the genome. A key epigenetic mechanism is the post-translational modification (PTM) of histone proteins.<sup>(4)</sup> The N-terminal tails of histones are heavily modified with numerous chemical moieties, including methylation, acetylation and phosphorylation.<sup>(5)</sup> Histone PTMs affect gene expression by controlling the transcription machinery's access to DNA and by recruitment of transcription factors.<sup>(6)</sup> These modifications comprise a 'histone code' that other proteins can 'write,' 'erase' and 'read.'<sup>(7)</sup> In the case of histone acetylation, histone acetyltransferases (HATs) are responsible for installing these groups while histone deacetylases (HDACs) remove them.<sup>(8)</sup>

HDAC inhibition has arisen as a potential strategy for treating ALS/FTD and other neurodegenerative diseases. For instance, the HDAC inhibitor Scriptaid can clear SOD1 aggregates *in vitro*.<sup>(9)</sup> Moreover, pan-HDAC inhibitors are neuroprotective in TDP-43 ALS models.<sup>(10)</sup> Similarly, phenylbutyrate, a general HDAC inhibitor, improves clinical phenotypes in an SOD1 ALS mouse model.<sup>(11)</sup> Trichostatin A (TSA), a pan-class I HDAC inhibitor, decreases motor neuron death in the same mouse model.<sup>(12)</sup>

Yeast provides an expedient, cost-efficient model to study certain aspects of neurodegeneration. FUS overexpression in yeast recapitulates several features of ALS/FTD pathology including FUS mislocalization and aggregation.<sup>(13)</sup> Furthermore, in contrast to mammalian cell models, yeast FUS ALS/FTD models display overt cellular demise allowing for convenient exploration of chemical tools to prevent cell death. Recent evidence

has tied FUS proteinopathy to the epigenome. FUS overexpression in yeast is associated with alterations to the histone PTM landscape.<sup>(8,14)</sup> In particular, FUS overexpression is connected to decreases in the levels of acetylation on Lysines 14 and 56 of Histone H3.<sup>(14)</sup> Histone PTMs are accessible pharmacological targets, and thus we speculated that chemical interventions aimed at modulating relevant epigenetic changes might lead to improved cell survival. Treatment with an HDAC inhibitor, such as TSA, could preserve histone acetylation levels by preventing the removal of acetyl groups from histone tails.

To test if TSA can ameliorate FUS proteinopathy, we measured the growth of yeast overexpressing FUS or a vector control in various concentrations of the drug. DMSO was used as a vehicle control. Serial yeast dilutions were grown on selective media supplemented with either glucose or galactose. Glucose suppresses FUS expression, while galactose activates it. Both FUS and vector control strains grew well on glucose, regardless of drug condition (Figure 1). Hence, TSA does not appear to impact yeast growth at the concentrations used. As previously reported,<sup>(13,14)</sup> FUS overexpression leads to a dramatic decrease in cell growth compared to a vector control (Figure 1). Excitingly, TSA ameliorated growth suppression in FUS yeast. TSA-treated FUS yeast grew better than untreated and vehicle control (Figure 1, black arrows). To semi-quantitatively measure the effect of TSA on yeast growth in the context of FUS overexpression, we assessed the image density of the middle spot from each growth condition and compared it to the corresponding spot in the vector control. Yeast overexpressing FUS treated with 2.50  $\mu$ M TSA grew significantly better than untreated or DMSO-treated yeast (Figure 1). Our results establish that interfering with HDAC activity via TSA treatment ameliorates the growth suppression associated with FUS overexpression in yeast.

We verified that the reduction of growth suppression did not result from changes in FUS levels or aggregation. There was no significant difference in FUS expression in any of the treatment conditions (Figure S1a). We confirmed that TSA did not affect FUS aggregation (Figure S1b and Figure S1c). Additionally, we established that TSA did not impact FUS aggregates or their cellular distribution (Figure S2). TSA and other HDAC inhibitors have been previously shown to trigger expression of Hsp70 which helps clear FUS aggregates and reduce proteotoxic stress.<sup>(15,16)</sup> However, as we see no reduction in FUS aggregation, we suspect the toxicity mitigation we observe does not involve proteostasis enhancement.

To clarify if the rescue elicited by TSA is FUS-specific and not generally applicable to misfolded proteins, we treated yeast overexpressing TDP-43 with TSA. As shown previously,<sup>(14,17)</sup> TDP-43 overexpression is very toxic (Figure S3, left panel). However, in contrast to FUS, TDP-43 proteinopathy is not linked to histone H3 acetylation disturbances.<sup>14</sup> TDP-43 yeast treated with 2.50  $\mu$ M TSA did not display growth improvement (Figure S3, right panel) suggesting that the effect of TSA is specific to FUS proteinopathy. We verified TSA did not impact TDP-43 expression levels (Figure S4a).

To probe whether detoxification occurs through epigenetic mechanisms, we asked if TSA restored levels of H3K14ac and H3K56ac. As previously reported,<sup>(14)</sup> untreated and DMSO-treated yeast displayed significantly decreased H3K14ac and H3K56ac compared to untreated vector control. Remarkably, TSA-treated FUS yeast showed a significant increase

in H3K14ac levels compared to untreated and DMSO-treated FUS yeast (Figure 2a). Surprisingly, TSA-treated yeast also showed significantly higher H3K56ac levels (Figure 2b). H3K56 is deacetylated by Hst3 and Hst4, which are insensitive to TSA.<sup>(18,19)</sup> However, acetylation of H3K56 is mediated by the lysine acetyltransferase Rtt109 in concert with the chaperones Vsp75 and Asf1.<sup>(20,21)</sup> Interestingly, H3K14ac drives Rtt109 to acetylate H3K56ac via Asf1 binding.<sup>(21)</sup> Through this crosstalk mechanism, it is possible that H3K14ac recovery is promoting the rise in H3K56ac levels.

TSA increases global acetylation levels in yeast, but at concentrations higher than those used in this study.<sup>(22,23)</sup> To verify that 2.50  $\mu$ M TSA did not affect acetylation at modification sites uncoupled from FUS proteinopathy, we measured H4K16ac levels (Figure S5). TSA did not increase H4K16ac, suggesting that 2.50  $\mu$ M TSA restores acetylation only for those modification sites originally impacted by FUS overexpression. To further investigate if 2.50  $\mu$ M TSA raised acetylation levels outside of the context of FUS proteinopathy, we probed for H3K14ac and H3K56ac in vector control yeast, where the levels of these PTMs are not decreased. TSA has no significant effect on H3K14ac or H3K56ac levels in vector control yeast (Figure S6). We also verified TSA did not impact H3K14ac or H3K56ac levels in TDP-43 yeast (Figure S4b and S4c).

As we observed evidence of histone PTM crosstalk, we wondered if other non-acetyl histone modifications connected to FUS proteinopathy would recover in response to TSA. FUS yeast, regardless of treatment conditions, retained reduced H3S10ph and H4R3me2asym levels (Figure S7). This suggests that the decreases in these PTMs are independent of histone acetylation. Furthermore, it establishes that the amelioration of growth suppression elicited by TSA is not related to changes in the levels of H3S10ph or H3Rme2asym. Akin to histone acetylation, TSA did not affect neither H3S10ph nor H4R3me2asym in vector control yeast (Figure S8).

Collectively, we show that TSA relieves growth suppression in yeast overexpressing FUS. This mitigation of toxicity is accompanied by preservation of H3K14ac and H3K56ac levels, but not H3S10ph or H4R3me2asym levels. We did not observe any changes in FUS expression or aggregation with TSA treatment. To the best of our knowledge, this is the first report of its kind tying specific histone acetylation sites to improvements in ALS/FTD cell demise. Pan-histone acetylation has been shown to increase after HDAC inhibition, but specific residues were not implicated.<sup>(12,24)</sup> Furthermore, our data raises the novel hypothesis that the toxic effect of protein aggregation in neurodegeneration is at least in part related to its association with altered histone marks, a notion that is not well characterized in ALS/FTD. We hypothesize reduction of toxicity occurs through modulation of epigenetic pathways. Very recently, pathogenic FUS has been found to interact with nucleoporins and impede nucleocytoplasmic transport.<sup>(25)</sup> It is possible that defects in nucleocytoplasmic transport result in mislocalization of HATs, resulting in impaired H3K14ac and H3K56ac which could then lead to aberrant gene expression and ultimately cell death (Figure 2c). However, further investigation is needed to definitively establish such a mechanism.

In conclusion, our results highlight that changes in histone PTMs are closely associated with the cell demise elicited by FUS proteinopathy. We illustrate the ability to counter the

detrimental effects of protein aggregation on cell survival by modulating specific histone modification changes via chemical treatment. Our results allude to novel mechanistic networks involving epigenetics that will enable new treatments for ALS/FTD and other neurodegenerative diseases.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## ABBREVIATIONS

<b>ALS</b>	Amyotrophic Lateral Sclerosis
<b>FTD</b>	Frontotemporal Dementia
<b>FUS</b>	Fused in Sarcoma
<b>HAT</b>	Histone Acetyltransferase
<b>HDAC</b>	Histone Deacetylase
<b>PTM</b>	Post-translational Modification
<b>TSA</b>	Trichostatin A

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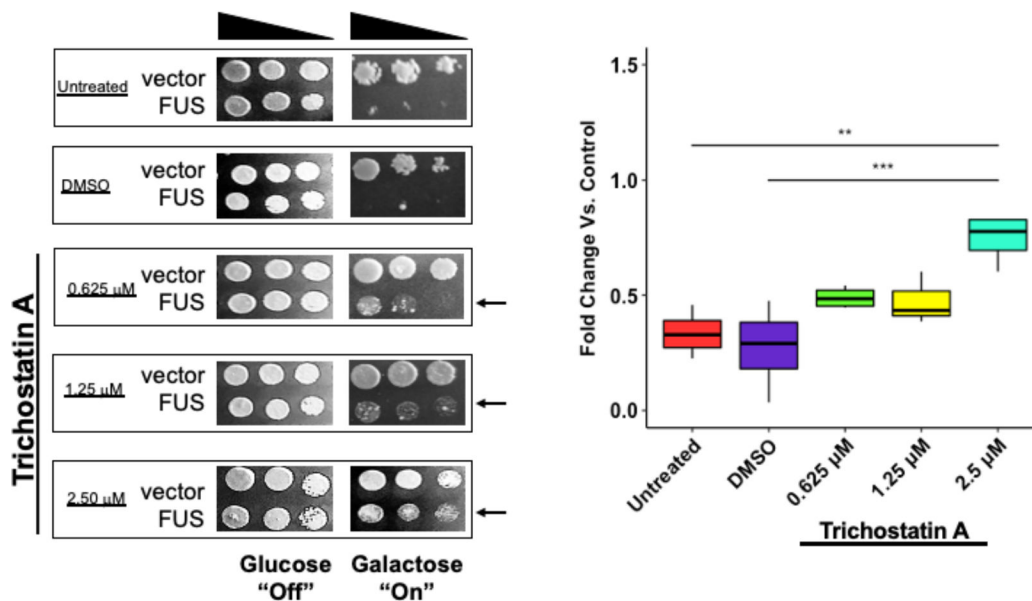
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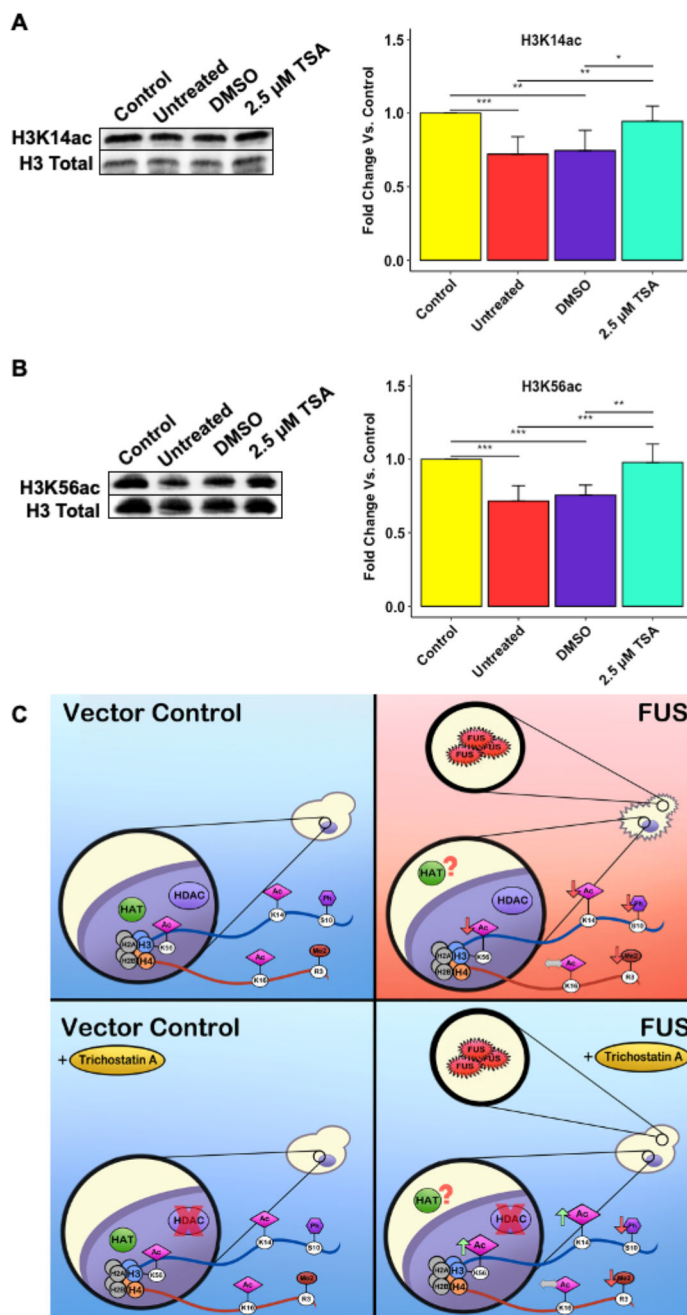
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**Figure 1. Trichostatin A relieves growth suppression in a FUS ALS/FTD yeast model.** Yeast expressing FUS or an empty vector were serially diluted 5-fold and spotted on glucose (off) or galactose (on) medium in the absence (untreated, DMSO) or presence of TSA at varying concentrations. Densitometric measurement of cell density compared to the untreated control is depicted. Box plot whiskers represent upper and lower quartiles. \*\*\* =  $p < 0.001$ , \*\* =  $p < 0.01$ .  $n = 4$ .





**Figure 2. Trichostatin A restores acetylation on Lysines 14 and 56 of Histone H3 in yeast FUS ALS/FTD models.**

Representative Western blots displaying the levels of H3K14ac (A,  $n = 6$ ) and H3K56ac (B,  $n = 6$ ) are shown. Histograms compiling multiple biological replicates are shown. (C) Putative mechanism for TSA effects in FUS ALS/FTD yeast models. Error bars represent  $+SD$ . \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .