Key Modulators of the Stress Granule Response TIA1, TDP-43, and G3BP1 are Altered by Polyglutamine Expanded ATXN7

Frida Niss

Stockholm University: Stockholms Universitet

Laura Piñero-Paez

Stockholms Universitet

Wajiha Zaidi

Stockholms Universitet

Einar Hallberg

Stockholms Universitet

Anna-Lena Ström (✉ anna-lena.strom@dbb.su.se)

Stockholms Universitet <https://orcid.org/0000-0002-8630-2127>

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Abstract

Spinocerebellar ataxia type ATXN7 (SCA7) and other polyglutamine (polyQ) diseases are caused by expansions of polyQ repeats in disease specific proteins. Aggregation of the polyQ proteins resulting in various forms of cellular stress, that could induce the stress granule (SG) response, is believed to be a common pathological mechanism in these disorders. SGs can contribute to cell survival, but have also been suggested to exacerbate disease pathology by seeding protein aggregation. In this study, we show that two SG related proteins, TDP-43 and TIA1, are sequestered into the aggregates formed by polyQ expanded ATXN7 in SCA7 cells. Interestingly, mutant ATXN7 also localises to induced SGs and this association altered the shape of the SGs. In spite of this, neither the ability to induce nor disassemble SGs, in response to arsenite stress induction or relief, was affected in SCA7 cells. Moreover, we could only detect a small, non-significant, increase in ATXN7 aggregation following SG induction. However, mutant ATXN7 expression in itself increased the speckling of the SG nucleating protein G3BP1 and the SG response. Taken together, our results indicate that the SG response is induced, and although some key modulators of SGs show altered behaviour, the dynamics appear normal in the presence of mutant ATXN7.

Introduction

Spinocerebellar Ataxia type 7 (SCA7) is a lethal neurodegenerative disease, which is part of a group of disorders termed polyglutamine (polyQ) diseases. The disorder is caused by an autosomal dominant mutation which results in the expansion of a glutamine repeat in the N-terminal of the Ataxin-7 (ATXN7) protein. Although ATXN7 is ubiquitously expressed, a subset of neurons in the central nervous system is especially affected [1], resulting in ataxia and blindness in the patient. ATXN7 normally functions within the deubiquitination (DUB) module of the STAGA/TFTC complex and participates in transcriptional regulation [2, 3]. Mutated ATXN7 can still incorporate into the STAGA/TFTC complex, but this interferes with the normal transcriptional function of the complex [4, 5]. In addition, STAGA/TFTC function is also disrupted by sequestration of the DUB module into protein aggregates formed by mutant ATXN7 [6, 7]. Moreover, apart from the normal interaction partners of ATXN7, a whole host of other proteins can also be found in the aggregates, all of whose loss likely could exacerbate the pathology, for review see [8]. Interestingly, a recent study using mass spectrometry to investigate the protein content of aggregates in a different polyglutamine disease, Huntington's disease (HD), found that an unexpectedly high proportion of the aggregate was made up of RNA binding proteins (RBPs) [9]. Furthermore, the RBPs that were sequestered into aggregates disproportionately often contained Prion like domains (PrLDs), indicating that the expanded polyglutamine domain of huntingtin, the protein mutated in HD, readily interacts with PrLD containing proteins and sequesters them into aggregates. In line with this, in SCA7 disease models and patients, several RBPs have also been found to localise to ATXN7 aggregates [10, 11].

As RBPs have many RNA and protein interaction partners and regulate RNA processing at several different steps [12], numerous important functions could be disturbed by interference of RBPs. In fact, mutations in several RBPs, including FUS and TDP-43, can cause neurodegenerative diseases [13–15].

The causative mutations in these two specific RBPs are often found in the C-terminus that controls the nuclear/cytoplasmic shuttling of the protein, resulting in cytoplasmic accumulation of the respective RBP. This indicates that mutations of FUS and TDP-43 could cause a loss-of-function effect in the nucleus, or a gain-of-function effect in the cytoplasm. It has been observed that mislocalisation of FUS and TDP-43 to the cytoplasm dysregulates some of their RNA targets [11, 16], and also can result in pathological cytoplasmic granule assemblies [17]. Thus, mutations that cause mislocalisation of these RBPs seem to result in tandem loss-of-function and gain-of-function effects. Since these effects are evidently pathogenic enough to bring on cell death and neurodegeneration in other neurodegenerative diseases, we hypothesised that sequestration-mediated dysregulation of RBPs should be able to exacerbate SCA7 pathology. Indeed, in a previous study, we could show that transcripts normally controlled by the RBP FUS, were dysregulated in a stable inducible SCA7 cell model expressing expanded ATXN7 [11]. Furthermore, since FUS is also involved in DNA repair [18], we were not surprised to find that there was an induction of DNA damage in mutant ATXN7 expressing cells as well. This contributed to the already ascertained list of various negative effects produced by expanded ATXN7 in cells, such as oxidative stress and disruptions of the proteasome and autophagy systems [19–21]. To cope with these types of stresses, cells can induce a specific type of response called stress granules (SGs), which are cytoplasmic, membrane-less organelles formed by RBPs and stalled polysomes through a controlled oligomerisation process [22]. These organelles are believed to increase cell survival by sorting mRNAs, allowing translation of necessary transcripts, while inhibiting translation of unnecessary RNAs, as well as regulating various signalling pathways and apoptosis during stress [23]. Following stress relief, the SGs should rapidly disassemble, restoring translation of all RNA transcripts. However, formation of SGs has also been suggested to seed the formation of pathological protein aggregates in several neurodegenerative diseases and thereby worsen pathology [23].

In light of the many mentioned similarities between neurodegenerative diseases when it comes to molecular stress pathology, we were eager to investigate the role of SGs in SCA7, a disease in which SGs have not been studied specifically. We could show that mutant ATXN7 induces the SG response and that two SG related RBPs, TDP-43 and TIA1, are sequestered into mutant ATXN7 aggregates. Despite this, SG assembly and disassembly in response to arsenite treatment was not affected in SCA7 cells. Interestingly, mutant ATXN7 was shown to co-localise with and affect the shape of the induced SGs, and this was accompanied by a small, though non-significant increase of mutant ATXN7 aggregation.

Results

TDP-43 co-localises with cytoplasmic ATXN7 aggregates

To investigate if stress granules (SGs), that are nucleated by PrLD containing RBPs [24, 25], are affected by the expression of expanded ATXN7 we used an inducible PC12 cell model expressing human full-length ATXN7 with either 10 (ATXN7Q10) or 65 (ATXN7Q65) glutamines to simulate SCA7 pathology. Both constructs were fused to GFP in the C-terminal. After 12 days of induction the ATXN7Q65 cells show advanced cytoplasmic and nuclear aggregate formation, as well as an increase in cytotoxicity, as

characterised in previous papers [19, 26, 20]. In comparison, the ATXN7Q10 induced cells show a strong but diffuse nuclear localisation of ATXN7, and no cytotoxicity (Fig. 1a).

First, we analysed if TDP-43, a PrLD containing RBP protein shown to participate in SG formation [27] and heavily implicated in other neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) [28, 29] and Alzheimer's disease (AD) [30, 31] was affected by mutant ATXN7. Through immunofluorescence we could observe a high co-localisation of TDP-43 with ATXN7 aggregates in the cytoplasm, though only 5% of nuclear ATXN7 aggregates contained TDP-43 (Fig. 1b-d). Our images also indicated a slight non-significant increase of total TDP-43 intensity after induction of ATXN7Q65 (Fig. 1e). It has previously been noted that ATXN7Q10 and ATXN7Q65 is cleaved after induction in our cell model, and while the non-cleaved protein remains mostly nuclear, the shorter polyQ tract containing N-terminal fragments are mainly found in the cytoplasm [26]. Based on the higher co-localisation with cytoplasmic aggregates, we therefore suspect that the PrLD of TDP-43 is more prone to interact with the exposed polyQ domain in N-terminal fragments of expanded ATXN7 than the full-length protein.

TDP-43 is hyperphosphorylated in ATXN7Q65 induced cells

Hyperphosphorylated TDP-43 has previously been observed in cell culture during polyglutamine expression [32], as well as in several other neurodegenerative diseases [33, 34], and has been identified as a pathological marker [35]. Therefore, we wanted to investigate if TDP-43 hyperphosphorylation occurred in our SCA7 cell model as well. Using western blots, we analysed the expression of TDP-43 after induction of ATXN7Q10 and ATXN7Q65 (Fig. 2). While there was no change in overall TDP-43 protein expression (Fig. 2b), we were in fact able to observe a significant increase in phosphorylated TDP-43 at serine 409/410, using two separate antibodies, when ATXN7Q65 was induced (Fig. 2c and e). We observed a similar trend in cells induced to express ATXN7Q10, though the variation between samples was too high for any conclusions to be drawn.

TIA1 co-localises with ATXN7 aggregates, while G3BP1 does not

TDP-43 can regulate the expression of two nucleating stress granule proteins, TIA1 and G3BP1 [27]. Since TDP-43 was hyperphosphorylated and sequestered into ATXN7 aggregates in SCA7 cells, we therefore next analysed the effect of expanded ATXN7 on TIA1 and G3BP1 localisation and expression. While TIA1 contains a PrLD, G3BP1 does not (PLAAC predictor [36]). As such, we expected there to be a higher chance of interaction between polyQ aggregates and TIA1, than with G3BP1. Indeed, when we immunostained TIA1 together with ATXN7 in induced PC12 cells, we saw that the weak TIA1 staining increased and condensed into the ATXN7 aggregates upon ATXN7Q65 induction (Figure 3a-b). In fact, TIA1 intensity peaks were observed in as many as 80% of cytoplasmic aggregates, and 60% of nuclear aggregates (Fig. 3c-d). However, filter trap assays could not capture TIA1 in the solubilised aggregated material (Figure 4a), suggesting that the interaction between the two proteins could not withstand harsh denaturing conditions. To be able to stain ATXN7, TIA1 and G3BP1 together with a DNA stain, we then used a different primary antibody against TIA1 as well (Fig. 3e). Using this antibody we could see the

same trend of increased TIA1 immunofluorescence in ATXN7Q65 induced cells (Fig. 3f). However, a similar increase could also be observed in ATXN7Q10 induced cells. Similarly, an increase in G3BP1 could also be observed in both induced ATXN7Q10 and ATXN7Q65 cells, though the increase was only significant in cells induced to express the mutant protein (Fig. 3g). As expected, when we analysed the ATXN7Q65 aggregates for TIA1 and G3BP1 co-localisation, we observed that while it was common for TIA1 to co-localise with the aggregates in both the cytoplasm and nucleus, there was virtually no co-localisation of G3BP1 with the aggregates (Fig. 3h-i). Neither could we find G3BP1 in the filter trap of solubilised aggregated material from ATXN7Q65 induced cells (Fig. 4a).

G3BP1 exhibits speckling behaviour in ATXN7Q65 induced cells

When staining for the various stress granule proteins, we noticed that although G3BP1 did not co-localise with ATXN7Q65 aggregates, the G3BP1 signal appeared different in the mutant cells. Using heat maps and surface plots, we could visualise this difference and show that the G3BP1 signal was more granulated in ATXN7Q65 induced cells, compared to non-induced cells or ATXN7Q10 induced cells (Fig. 5a-c). ATXN7Q10 induced cells did show higher intensity peaks than non-induced cells as well though. Therefore, we wanted to further analyse and better quantify the G3BP1 behaviour. For this we used a CellProfiler pipeline to assay variance, determining the texture of the signal. Indeed, we found a 4-fold increase in texture of the G3BP1 signal in ATXN7Q65 induced cells compared to non-induced controls and ATXN7Q10 induced cells (Fig. 5d). This led us to believe that the stress induced by ATXN7Q65 expression is causing G3BP1 to start exhibiting speckling behaviour, preparing for stress granule formation. In fact, when quantifying the few stress granule like speckles that were formed by G3BP1 in our non-induced or induced cells, we found an approximate 6-fold increase of speckles in ATXN7Q65 induced cells compared to non-induced cells (Fig. 5e), though no such striking difference was found in ATXN7Q10 induced cells.

Stress granule formation is enhanced by expanded ATXN7

In light of the effect ATXN7Q65 induction had on TIA1 and G3BP1, two important nucleators of SGs, next we investigated SG formation in our PC12 SCA7 cell model. As G3BP1 did not co-localise with ATXN7Q65 aggregates, we used mainly this protein to assay stress granule formation. To induce robust SG formation we used arsenite, a compound causing oxidative stress. After one hour of arsenite treatment, we could ascertain that PC12 cells readily formed G3BP1, TIA1, as well as double positive (defined as SGs) granules (Fig. 6a-b). ATXN7Q65 induced cells also produced aggregates as usual, that were distinguished from the SGs by shape, size and G3BP1 content. As pointed out in figure 6a (thin arrows), we could still see TIA1, but not G3BP1, co-localising with mutant ATXN7Q65 aggregates during arsenite stress conditions. However, TIA1 was also readily observed to co-localise with G3BP1 granules, and thus seems to be able to participate in SG formation despite sequestration into ATXN7Q65 aggregates. Thus, the formation of SGs does not appear to be inhibited by ATXN7Q65 expression. In fact, in agreement with the behaviour of G3BP1 in non-arsenite stressed cells, the stress caused by expanded ATXN7 seems to cause a trend towards more G3BP1, TIA1 as well as G3BP1-TIA1 double positive granules (Fig. 6b). The

smaller trend towards more granules in ATXN7Q65 induced cells after arsenite treatment, compared to the significantly higher G3BP1 speckling before additional stress application, may be because the upper cellular limit of how many SGs can be formed is approached with arsenite treatment, thus reducing the observable difference between control and mutant cells. To further investigate if mutant ATXN7 results in a stronger SG response, human fibroblasts from a young SCA7 patient, or an apparently healthy control individual of similar age, were investigated. Consistent with our PC12 data, the SCA7 fibroblasts showed an increased number of G3BP1 and TIA1 positive granules compared to the control fibroblasts, upon arsenite treatment (7a-b). Taken together, these data indicate that expansion of ATXN7 causes stress that triggers the SG response.

Expanded ATXN7 co-localises with arsenite induced G3BP1 granules and affects their shape

Although we could not observe G3BP1 co-localising to ATXN7 aggregates in non-stressed cells (Fig. 3), a clear co-localisation of mutant ATXN7 to a subset of G3BP1/TIA1 double positive stress granules could be observed upon arsenite treatment (Fig. 6a, thick arrows). Moreover, throughout imaging of arsenite treated cells, we noticed that the shape of G3BP1 granules was different in non-induced controls compared to in ATXN7Q65 induced cells. While controls exhibited more elongated G3BP1 granules, ATXN7Q65 induced cells showed more rounded granules (Fig. 6c). This was confirmed by measuring the eccentricity (Fig. 6d), as well as the formfactor (Fig S2) of the G3BP1 granules in CellProfiler. Neither the area nor the perimeter were significantly different in the two granule populations (Fig S1).

Surprisingly, when imaging using a confocal microscope to capture the weaker signal from the endogenous rat ATXN7, we found that endogenous rat ATXN7 also could co-localise with arsenite induced G3BP1 granules (Fig. 6e). When quantifying the co-localisation of the G3BP1 positive granules with ATXN7 positive granules through immunofluorescence, there was a clear overlap of the two signals after treatment with arsenite, both with endogenous rat ATXN7, and overexpressed human ATXN7Q10/Q65 (Fig. 6f). Consistent with this we could detect the occurrence of human endogenous ATXN7 in SGs in SH-SY5Y cells and control human fibroblasts, although the association was weaker in these cells (Fig S2 and Fig. 7a, 7c-e). In contrast, a stronger overlap between ATXN7 and G3BP1/TIA1 could be observed in human SCA7 fibroblasts (Fig. 7a, c-e). Taken together, these data indicate that wild-type ATXN7 may interact with SGs, and that this interaction might become stronger upon repeat expansion. Association with SGs have been speculated to seed polyglutamine protein aggregation [23]. In spite of this, we could not observe any increase in mutant ATXN7 aggregates or fusion between SGs and aggregates during our immunofluorescence experiments (data not shown). However, the signal intensity of insoluble ATXN7 in the filter trap assay did show a trend towards more aggregated ATXN7 upon SG induction with arsenite (Fig. 4b).

Expanded ATXN7 does not interfere with stress granule recovery

Upon stress alleviation, SGs should rapidly disassemble. However, the presence of aggregating, pathological proteins in SGs have been proposed to potentially prevent this process [23]. We were therefore curious to see if SG clearance following recovery from stress, i.e. arsenite treatment, was affected by ATXN7Q65. To analyse this, induced or non-induced PC12 cells were treated with arsenite for one hour, allowed to recover from the treatment for 0-120 minutes, and the number of G3BP1 positive granules determined at different time points (Fig. 8a-b). Surprisingly, no difference in the SG disassembly rate could be observed between ATXN7Q65 induced cells and non-induced control cells (Fig. 8c). When we repeated the experiment with inclusion of TIA1 staining along with G3BP1 staining, we received the same results (Fig. 8d). Therefore, we conclude that although expanded ATXN7 can localise to SGs, this does not affect the clearance rate of stress granules.

Discussion

Stress granules represent a conserved component of the cellular response to stress and are crucial for cell survival [23]. In the current study, we have shown that expanded ATXN7 affects the expression and/or behaviour of TDP-43, TIA1 and G3BP1, three RNA-binding proteins involved in the stress granule response. We could show that phosphorylation of S409/S410 in TDP-43 is increased and that TDP-43 co-localises with ATXN7 aggregates in SCA7 cells. This is consistent with previous studies identifying increased TDP-43 phosphorylation and sequestration in SCA7 mice and patients, as well as in several other neurodegenerative diseases [37–41, 34]. Findings in other diseases have indicated that TDP-43 exits the nucleus during stress, [42, 27] and that TDP-43 phosphorylation exacerbates TDP-43 related neuropathology [35, 43]. However, while Coudert et al. found that TDP-43 phosphorylation was secondary to aggregation, Hasegawa et al. observed that TDP-43 is more prone to multimerise into filaments after phosphorylation by Casein kinase 1 [39, 41]. Interestingly, we found a higher co-localisation of TDP-43 with cytoplasmic, rather than nuclear, ATXN7 aggregates. Therefore, we hypothesise that the stress caused by expanded ATXN7 in our SCA7 cell model could cause TDP-43 to exit the nucleus, interact with cytoplasmic polyQ fragments, and become phosphorylated, which strengthens the interaction and causes accumulation of TDP-43 into aggregates.

Besides TDP-43, we could also observe a clear co-localisation of TIA1 with both nuclear and cytoplasmic ATXN7 aggregates. This together with the fact that TDP-43 has been shown to positively regulate the expression of G3BP1 [44, 27], initially led us to expect decreased soluble levels of TDP-43, TIA1 and G3BP1 in our SCA7 cell model. However, western blot and IF analyses instead revealed similar or statistically significantly increased expression level of these RBPs, suggesting that the stress caused by mutant ATXN7 triggers the stress granule response pathway. As a matter of fact, increased expression of stress granule nucleating proteins has previously been observed in Huntington's disease patients and models [45, 46]. Not only were we able to observe an increased level of G3BP1 in our expanded ATXN7 expressing cells, but we also found that the behaviour of G3BP1 changed. Although fully formed G3BP1 positive SGs were rare in the SCA7 cells, G3BP1 displayed a statistically significant increase in signal texture and speckling, indicating condensation of G3BP1 into pre-SG structures in ATXN7 cells. Furthermore, following treatment with the SG inducing compound arsenite, a trend towards more SGs in

mutant ATXN7, versus control cells, could also be observed. The fact that the arsenite induced effect on SG formation in mutant versus control cells was smaller than the observed difference in G3BP1 speckling levels before arsenite treatment, is most likely due to the close to maximal induction of the SG response by arsenite, as mentioned previously. Hence, despite the sequestration of TDP-43 and TIA1, the ability of SCA7 cells to induce SGs is not reduced and in fact mutant ATXN7 appears to induce the SG response.

Although we could not see any sequestration of G3BP1 into mutant ATXN7 aggregates, a clear co-localisation of mutant ATXN7 to SGs could be observed upon arsenite mediated induction of stress granules in both SCA7 PC12 cells and patient fibroblasts. Surprisingly, endogenous and transgenic wild-type ATXN7 also localised to SGs to some extent. To our knowledge this is the first time ATXN7 has been linked to SGs, though another STAGA DUB subunit, *sus1*, has been shown to localise to SGs in yeast [47]. Moreover, the yeast ATXN7 ortholog *sgf73*, along with the STAGA DUB module, were recently linked to export of essential transcripts in response to stress [48]. Further studies to investigate the connection between wild-type ATXN7, the STAGA DUB module, and the stress granule response would thus be interesting.

Despite both endogenous and mutant ATXN7 localising to SGs, we could observe a difference in the shape of the SGs when comparing expanded ATXN7 expressing cells to non-induced control cells, indicating that expanded ATXN7 does not interact with SGs identically to wild-type. An interesting recent paper has compared SG structure after overexpression of several SG proteins, such as TIA1, G3BP1, TDP-43, FMR1 etc. [49]. They noted that when different SG proteins were overexpressed to induce SG formation, granules of two classes were formed, which they designated as rough and smooth granules. G3BP1 and TDP-43 overexpression resulted in smooth granules, while increased TIA1 levels produced rough granules. While we can speculate that the partial sequestration of TDP-43 and/or TIA1 into polyQ aggregates, as well as the co-localisation of mutant ATXN7 with stress granules could affect granule shape and function, it is important to note that SGs are incredibly complex structures. The nucleation is performed by numerous proteins and mRNAs [50], and the resulting granule is composed of both a stable core and a more dynamic shell [51]. It is therefore difficult to predict how small subtractions or additions of specific proteins will affect shape or function of SGs. In fact, from our results it is clear that stress granule induction, as well as disassembly do not appear to be negatively affected by mutant ATXN7 expression. Furthermore, SGs have been suggested to act as platforms able to nucleate polyQ aggregation [52, 23]. However, we could not observe any statistical increase in ATXN7 aggregation following SG induction, although there was a trend towards increased insoluble ATXN7 levels captured by filter trap. Nevertheless, considering that SCA7 is a chronic disease and the SG response is presumably continuously activated in SCA7 patients as a result of the DNA damage, oxidative stress and overloaded proteasome/autophagy systems, that can be observed in disease models [11, 19–21], it is plausible that SGs in the long run could potentiate polyQ aggregation. In fact, SGs formed in response to chronic stress have been suggested to promote cell death, rather than survival, for review see [53]. On the other hand, the long-term accumulation of toxic expanded ATXN7 species [54, 1, 55], could with time result in sufficient disruption of PrLD containing RBPs, both potentially within SGs and elsewhere, resulting in deleterious dysfunctions of the stress response.

In conclusion, we find that although the stress granule proteins TIA1 and TDP-43 are sequestered into mutant ATXN7 aggregates, this does not inhibit stress granule formation or disassembly. In contrast, a heightened condensation behaviour of G3BP1 and trend towards more stress granules could be observed in SCA7 cells, suggesting that expansion of ATXN7 induces stress that triggers the SG response. Most interestingly, it seems that ATXN7 localises to SGs and that association of the mutant protein into SGs, alters the shape of the SGs. The role of this association, as well as the molecular consequences of an altered SG shape in SCA7 disease, needs to be clarified in further studies.

Materials And Methods

Cell Culture

All cells were cultured at 37°C and 5% CO₂ in a humidified environment. PC12 cells stably transfected with the inducible Tet-Off system controlling ATXN7 expression [26] were cultured in Dulbecco's Modified Eagle Medium (Gibco, #41966-029), supplemented with 10% Horse serum (Gibco, #16050-122), 5% Tetracycline-screened fetal bovine serum (FBS) (Thermofisher Scientific, #SH30070.03), 1% PEST (Gibco, #15140-122), 0.1% G418 (Gibco, #11811031), 100 µg/ml Hygromycin (Life technologies, #10687010) and 1 µg/ml Doxycycline (Merck, #D9891-5G). For induction of ATXN7 expression, doxycycline was removed from the medium for 12 days. SHSY-5Y cells were cultured in Minimum Essential Medium (MEM) (Gibco, #21090-022), 10% FBS (Gibco, #10270-106), 1% Non-essential amino acids (NEAA) (Gibco, #11140035), 1% L-glutamine (Gibco, #25030024) and 1% PEST. Fibroblasts (Coriell institute, #GM03561 and #GM07492) were cultured in MEM supplemented with 20% FBS, 1% NEAA, 1% L-glutamine and 1% PEST.

Immunofluorescence

Prior to staining, cells were grown on no. 1.5 coverslips coated with 0.5% gelatin. After induction and/or treatment with 0.5 mM Arsenite for one hour, the cells were rinsed once in PBS and then fixed with 4% PFA for 45 minutes in room temperature. This was followed by 3x10 minutes in PBST (0.1% Triton-X100 in PBS), 30 minutes of blocking (10% FBS in PBST), and incubation over night with primary antibodies in primary antibody solution (1% FBS in PBST). The primary antibodies employed in this study were: rabbit α-7 [1, 56] (1:500), mouse α-TDP-43 (Abcam, #ab104223, 1:1000), mouse α-TIA1 (Santa Cruz, #sc-166247, 1:250), goat α-TIA1 (Abcam, #ab61700, 1:200), and mouse α-G3BP1 (ProteinTech, #66486-1, 1:200). After the primary antibody incubation the coverslips were washed 3x10 minutes in PBST, then incubated with secondary antibodies (Alexafluor 488 α-rabbit/568 α-mouse/568 α-goat/633 α-mouse, Thermofisher Scientific, 1:5000) in blocking solution for one hour. 5 µM DRAQ5 (Thermofisher Scientific, #62251) or 5 µg/ml Hoechst (Sigma-Aldrich) were added as DNA markers to the secondary antibody solutions. The coverslips were then washed 3x10 minutes in PBST, mounted with Fluoromount-G (Southern Biotech, #0100-01) on microscope slides, and sealed with nail polish.

Image Acquisition and Analysis

3-channel images were captured using a Zeiss axiovert 200 multipoint confocal microscope with a 63x/1.4 NA oil immersion objective and the Micromanager open source software [57]. 4-channel images and images of fibroblasts were captured using an Axio observer ATXN7 widefield microscope with a 100x/1.4 NA oil immersion objective and the Zen Blue software (Zeiss). Representative images were handled in FIJI open source software [58], and brightness/contrast settings were constant for each set of representative images, as indicated in the figure legends. This resulted in diffuse or endogenous staining displayed being weak compared to the various aggregate and speckle stainings. For analysis of aggregate co-localisation with TDP-43, TIA1 or G3BP1, the images were scored as follows. Five cytoplasmic and five nuclear aggregates from five randomly selected images in each replicate were assessed. Through each aggregate a line was drawn using the FIJI software. The intensity profiles of each stain was compared to the ATXN7 channel, and if there was a coinciding intensity peak 2-fold higher than the immediate background staining, the aggregate was scored as positive for co-localising stain. Heatmaps and surface plots showcasing the G3BP1 staining were created using the 'Lookup Table: royal' and 'Surface Plot' functions in FIJI. The total intensity of each image was measured using the open source software CellProfiler [59] and normalised to the number of nuclei in the image, counted manually or automatically. Likewise, CellProfiler was employed to assess the number of G3BP1 speckles, TIA1 speckles, ATXN7 speckles and stress granules after arsenite induction, while the number of G3BP1 speckles in non-stressed cells were counted manually according to the following rules. The speckle must be shaped like an arsenite-induced speckle, i.e. rounded or oval-shaped, and the G3BP1 staining intensity must be 8-fold higher compared to immediate background. For automatic speckle counting, the images were thresholded using a global Otsu threshold with three classes assigning the middle class to background. The minimum threshold was set for each replicate according to arsenite treated non-induced PC12 cells. Additionally, CellProfiler was used to measure texture of the G3BP1 staining from confocal images, as determined using the variance output, as well as shape and size of G3BP1 speckles, as determined using the eccentricity, formfactor, area, and perimeter outputs.

Western blots

Prior to harvest of protein extracts, the cells were seeded in 10 cm petri dishes. Following induction for 12 days, cells were rinsed once with ice-cold PBS and then lysed with RIPA buffer containing a protease inhibitor cocktail for 15 minutes. Cells were then collected using cell scrapers and centrifuged at 4°C and 20 000×g for 10 minutes. The pellet was discarded and the protein concentration of the supernatant was determined using a Bradford assay. 6×Loading dye containing SDS was added to each sample before they were boiled for 10 minutes at 95°C, vortexed and loaded onto a 8% polyacrylamide gel using apparatus from Bio-Rad. After electrophoresis, the samples were blotted onto a 0.2 µm nitrocellulose membrane. The membrane was rinsed with PBS and blocked for one hour in 10% dry milk powder in TBST (100 mM Tris-buffered saline pH 7.4 and 0.1% Tween-20). Following blocking the membrane was incubated over night at 4°C with primary antibody in 2% dry milk in TBST, after which it was rinsed 3x10 minutes in TBST, incubated with secondary antibody (1:50 000, HRP-conjugated, Invitrogen) in 2% dry milk in TBST and rinsed 3x10 minutes with TBST again. The primary antibodies used were rabbit α-7 [1, 56] (1:700), mouse α-TDP-43 (Abcam, #ab104223, 1:10 000), rat α-TDP-43 (S409/S410) (Biolegend,

#829901, 1:500), rat α -TDP-43 (S409/S410) (Abcam, #ab184683, 1:1000), and mouse α -Tubulin (Sigma, #T9026, 1:1000). The secondary antibody was detected using the SuperSignalTM West Dura Extended Duration Substrate (ThermoFisher Scientific, #34075) and a Chemidoc XRS+ (Bio-Rad). Image analysis was performed using ImageLab software (Bio-Rad).

Filter traps

Cells were seeded into 10 cm petri dishes and induced prior to harvest. On the 12th day of induction, the cells were rinsed with ice-cold PBS, lysed with RIPA buffer supplemented with protease inhibitor cocktail and scraped into Eppendorf tubes. They were then centrifuged for 10 minutes at 4°C and 21 000×*g*. The supernatant was discarded and the pellet was washed twice with RIPA buffer before being re-suspended in DNase I containing buffer for one hour. The protein concentration of the pellet suspension was determined using a Bradford assay, and the samples were mixed with SDS and DTT (final concentrations 2% and 100 mM, respectively) and boiled for five minutes. A 0.2 μ m nitrocellulose membrane was loaded into a Dot-Blot manifold (Bio-Rad) and washed with 0.1% SDS solution before equal amounts of protein were added from each sample. The samples were vacuum-filtered through the membrane followed by two washes of 0.1% SDS solution. The membrane was then immunoblotted as described previously in the Western Blot section.

Declarations

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Competing Interests

The authors have no competing interests to declare that are relevant to the content of this article.

Author Contributions

Conceptualisation: Frida Niss, Einar Hallberg and Anna-Lena Ström; Methodology: Frida Niss, Laura Piñero-Paez and Wajiha Zaidi; Formal analysis and investigation: Frida Niss, Laura Piñero-Paez and Wajiha Zaidi; Writing – original draft preparation: Frida Niss; Writing- review and editing: Frida Niss, Laura Piñero-Paez, Wajiha Zaidi, Einar Hallberg and Anna-Lena Ström; Funding acquisition: Anna-Lena Ström; Supervision: Einar Hallberg and Anna-Lena Ström.

Data Availability

The data sets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

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Not applicable

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References

1. Jonasson J, Ström A-L, Hart P, Brännström T, Forsgren L, Holmberg M (2002) Expression of ataxin-7 in CNS and non-CNS tissue of normal and SCA7 individuals. *Acta Neuropathol* 104(1):29–37. doi:10.1007/s00401-001-0514-4
2. Köhler A, Zimmerman E, Schneider M, Hurt E, Zheng N (2010) Structural Basis for Assembly and Activation of the Heterotetrameric SAGA Histone H2B Deubiquitinase Module. *Cell* 141(4):606–617. doi:10.1016/j.cell.2010.04.026
3. Samara NL, Datta AB, Berndsen CE, Zhang X, Yao T, Cohen RE, Wolberger C (2010) Structural Insights into the Assembly and Function of the SAGA Deubiquitinating Module. *Science* 328(5981):1025–1029. doi:10.1126/science.1190049
4. Palhan VB, Chen S, Peng G-H, Tjernberg A, Gamper AM, Fan Y, Chait BT, La Spada AR, Roeder RG (2005) Polyglutamine-expanded ataxin-7 inhibits STAGA histone acetyltransferase activity to produce retinal degeneration. *Proc Natl Acad Sci USA* 102(24):8472–8477. doi:10.1073/pnas.0503505102
5. McMahon SJ, Pray-Grant MG, Schieltz D, Yates JR, Grant PA (2005) Polyglutamine-expanded spinocerebellar ataxia-7 protein disrupts normal SAGA and SLIK histone acetyltransferase activity. *Proc Natl Acad Sci USA* 102(24):8478–8482. doi:10.1073/pnas.0503493102
6. Yang H, Liu S, He W-T, Zhao J, Jiang L-L, Hu H-Y (2015) Aggregation of Polyglutamine-expanded Ataxin 7 Protein Specifically Sequesters Ubiquitin-specific Protease 22 and Deteriorates Its Deubiquitinating Function in the Spt-Ada-Gcn5-Acetyltransferase (SAGA) Complex. *J Biol Chem* 290(36):21996–22004. doi:10.1074/jbc.M114.631663
7. Lan X, Koutelou E, Schibler AC, Chen YC, Grant PA, Dent SYR (2015) Poly(Q) Expansions in ATXN7 Affect Solubility but Not Activity of the SAGA Deubiquitinating Module. *Mol Cell Biol* 35(10):1777–1787. doi:10.1128/MCB.01454-14
8. Yang H, Hu HY (2016) Sequestration of cellular interacting partners by protein aggregates: implication in a loss-of-function pathology. *FEBS J* 283(20):3705–3717. doi:10.1111/febs.13722

9. Wear MP, Kryndushkin D, O'Meally R, Sonnenberg JL, Cole RN, Shewmaker FP (2015) Proteins with Intrinsically Disordered Domains Are Preferentially Recruited to Polyglutamine Aggregates. *PLoS ONE* 10(8):e0136362. doi:10.1371/journal.pone.0136362
10. Alves S, Marais T, Biferi M-G, Furling D, Marinello M, El Hachimi K, Cartier N, Ruberg M, Stevanin G, Brice A, Barkats M, Sittler A (2016) Lentiviral vector-mediated overexpression of mutant ataxin-7 recapitulates SCA7 pathology and promotes accumulation of the FUS/TLS and MBNL1 RNA-binding proteins. *Mol Neurodegeneration* 11:58. doi:10.1186/s13024-016-0123-2
11. Niss F, Zaidi W, Hallberg E, Ström AL (2020) Polyglutamine expanded Ataxin-7 induces DNA damage and alters FUS localization and function. *Mol Cell Neurosci* 110:103584. doi:10.1016/j.mcn.2020.103584
12. Burd CG, Dreyfuss G (1994) Conserved structures and diversity of functions of RNA-binding proteins. *Science* 265(5172):615–621. doi:10.1126/science.8036511
13. Neumann M, Rademakers R, Roeber S, Baker M, Kretzschmar HA, Mackenzie IRA (2009) A new subtype of frontotemporal lobar degeneration with FUS pathology. *Brain* 132(11):2922–2931. doi:10.1093/brain/awp214
14. Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, Mann D, Tsuchiya K, Yoshida M, Hashizume Y, Oda T (2006) TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun* 351(3):602–611. doi:10.1016/j.bbrc.2006.10.093
15. Arai T, Mackenzie IRA, Hasegawa M, Nonaka T, Niizato K, Tsuchiya K, Iritani S, Onaya M, Akiyama H (2009) Phosphorylated TDP-43 in Alzheimer's disease and dementia with Lewy bodies. *Acta Neuropathol* 117(2):125–136. doi:10.1007/s00401-008-0480-1
16. Fuentealba RA, Udan M, Bell S, Wegorzewska I, Shao J, Diamond MI, Weihl CC, Baloh RH (2010) Interaction with Polyglutamine Aggregates Reveals a Q/N-rich Domain in TDP-43. *J Biol Chem* 285(34):26304–26314. doi:10.1074/jbc.M110.125039
17. Mackenzie IRA, Rademakers R, Neumann M (2010) TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia. *Lancet Neurol* 9(10):995–1007. doi:10.1016/S1474-4422(10)70195-2
18. Mastrocola AS, Kim SH, Trinh AT, Rodenkirch LA, Tibbetts RS (2013) The RNA-binding protein fused in sarcoma (FUS) functions downstream of poly(ADP-ribose) polymerase (PARP) in response to DNA damage. *J Biol Chem* 288(34):24731–24741. doi:10.1074/jbc.M113.497974
19. Ajayi A, Yu X, Wahlo-Svedin C, Tsirigotaki G, Karlström V, Ström A-L (2015) Altered p53 and NOX1 activity cause bioenergetic defects in a SCA7 polyglutamine disease model. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* 1847(4–5):418–428. doi:10.1016/j.bbabbio.2015.01.012
20. Yu X, Muñoz-Alarcón A, Ajayi A, Webling KE, Steinhof A, Langel Ü, Ström A-L (2013) Inhibition of Autophagy via p53-Mediated Disruption of ULK1 in a SCA7 Polyglutamine Disease Model. *J Mol Neurosci* 50(3):586–599. doi:10.1007/s12031-013-0012-x
21. Alves S, Cormier-Dequaire F, Marinello M, Marais T, Muriel M-P, Beaumatin F, Charbonnier-Beaupel F, Tahiri K, Seilhean D, El Hachimi K, Ruberg M, Stevanin G, Barkats M, den Dunnen W, Priault M, Brice A,

- Durr A, Corvol J-C, Sittler A (2014) The autophagy/lysosome pathway is impaired in SCA7 patients and SCA7 knock-in mice. *Acta Neuropathol* 128(5):705–722. doi:10.1007/s00401-014-1289-8
22. Kedersha N, Anderson P (2002) Stress granules: sites of mRNA triage that regulate mRNA stability and translatability. *Biochem Soc Trans* 30(6):963–969. doi:10.1042/bst0300963
23. Marcelo A, Koppenol R, de Almeida LP, Matos CA, Nobrega C (2021) Stress granules, RNA-binding proteins and polyglutamine diseases: too much aggregation? *Cell Death Dis* 12(6):592. doi:10.1038/s41419-021-03873-8
24. Gilks N, Kedersha N, Ayodele M, Shen L, Stoecklin G, Dember LM, Anderson P (2004) Stress granule assembly is mediated by prion-like aggregation of TIA-1. *Mol Biol Cell* 15(12):5383–5398. doi:10.1091/mbc.e04-08-0715
25. Tourriere H, Chebli K, Zekri L, Courselaud B, Blanchard JM, Bertrand E, Tazi J (2003) The RasGAP-associated endoribonuclease G3BP assembles stress granules. *J Cell Biol* 160(6):823–831. doi:10.1083/jcb.200212128
26. Yu X, Ajayi A, Boga NR, Ström A-L (2012) Differential Degradation of Full-length and Cleaved Ataxin-7 Fragments in a Novel Stable Inducible SCA7 Model. *J Mol Neurosci* 47(2):219–233. doi:10.1007/s12031-012-9722-8
27. McDonald KK, Aulas A, Destroismaisons L, Pickles S, Beleac E, Camu W, Rouleau GA, Vande Velde C (2011) TAR DNA-binding protein 43 (TDP-43) regulates stress granule dynamics via differential regulation of G3BP and TIA-1. *Hum Mol Genet* 20(7):1400–1410. doi:10.1093/hmg/ddr021
28. Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, McCluskey LF, Miller BL, Masliah E, Mackenzie IR, Feldman H, Feiden W, Kretzschmar HA, Trojanowski JQ, Lee VMY (2006) Ubiquitinated TDP-43 in Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis. *Science* 314(5796):130–133. doi:10.1126/science.1134108
29. Chou C-C, Zhang Y, Umoh ME, Vaughan SW, Lorenzini I, Liu F, Sayegh M, Donlin-Asp PG, Chen YH, Duong DM, Seyfried NT, Powers MA, Kukar T, Hales CM, Gearing M, Cairns NJ, Boylan KB, Dickson DW, Rademakers R, Zhang Y-J, Petrucelli L, Sattler R, Zarnescu DC, Glass JD, Rossoll W (2018) TDP-43 pathology disrupts nuclear pore complexes and nucleocytoplasmic transport in ALS/FTD. *Nat Neurosci* 21(2):228–239. doi:10.1038/s41593-017-0047-3
30. Uryu K, Nakashima-Yasuda H, Forman MS, Kwong LK, Clark CM, Grossman M, Miller BL, Kretzschmar HA, Lee VMY, Trojanowski JQ, Neumann M (2008) Concomitant TAR-DNA-Binding Protein 43 Pathology Is Present in Alzheimer Disease and Corticobasal Degeneration but Not in Other Tauopathies. *J Neuropathol Exp Neurol* 67(6):555–564. doi:10.1097/NEN.0b013e31817713b5
31. Amador-Ortiz C, Lin WL, Ahmed Z, Personett D, Davies P, Dara R, Graff-Radford NR, Hutton ML, Dickson DW (2007) TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. *Ann Neurol* 61(5):435–445. doi:10.1002/ana.21154
32. Coudert L, Nonaka T, Bernard E, Hasegawa M, Schaeffer L, Leblanc P (2019) Phosphorylated and aggregated TDP-43 with seeding properties are induced upon mutant Huntingtin (mHtt)

- polyglutamine expression in human cellular models. *Cell Mol Life Sci* 76(13):2615–2632.
doi:10.1007/s00018-019-03059-8
33. Hasegawa M, Arai T, Nonaka T, Kametani F, Yoshida M, Hashizume Y, Beach TG, Buratti E, Baralle F, Morita M, Nakano I, Oda T, Tsuchiya K, Akiyama H (2008) Phosphorylated TDP-43 in frontotemporal lobar degeneration and ALS. *Ann Neurol* 64(1):60–70. doi:10.1002/ana.21425
34. Neumann M, Kwong LK, Lee EB, Kremmer E, Flatley A, Xu Y, Forman MS, Troost D, Kretzschmar HA, Trojanowski JQ, Lee VM (2009) Phosphorylation of S409/410 of TDP-43 is a consistent feature in all sporadic and familial forms of TDP-43 proteinopathies. *Acta Neuropathol* 117(2):137–149.
doi:10.1007/s00401-008-0477-9
35. Liachko NF, Guthrie CR, Kraemer BC (2010) Phosphorylation promotes neurotoxicity in a *Caenorhabditis elegans* model of TDP-43 proteinopathy. *J Neurosci* 30(48):16208–16219.
doi:10.1523/jneurosci.2911-10.2010
36. Lancaster AK, Nutter-Upham A, Lindquist S, King OD (2014) PLAAC: a web and command-line application to identify proteins with prion-like amino acid composition. *Bioinformatics* 30(17):2501–2502. doi:10.1093/bioinformatics/btu310
37. Alves S, Marais T, Biferi MG, Furling D, Marinello M, El Hachimi K, Cartier N, Ruberg M, Stevanin G, Brice A, Barkats M, Sittler A (2016) Lentiviral vector-mediated overexpression of mutant ataxin-7 recapitulates SCA7 pathology and promotes accumulation of the FUS/TLS and MBNL1 RNA-binding proteins. *Mol Neurodegener* 11(1):58. doi:10.1186/s13024-016-0123-2
38. Arai T, Mackenzie IR, Hasegawa M, Nonaka T, Niizato K, Tsuchiya K, Iritani S, Onaya M, Akiyama H (2009) Phosphorylated TDP-43 in Alzheimer's disease and dementia with Lewy bodies. *Acta Neuropathol* 117(2):125–136. doi:10.1007/s00401-008-0480-1
39. Coudert L, Nonaka T, Bernard E, Hasegawa M, Schaeffer L, Leblanc P (2019) Phosphorylated and aggregated TDP-43 with seeding properties are induced upon mutant Huntingtin (mHtt) polyglutamine expression in human cellular models. *Cell Mol Life Sci* 76(13):2615–2632.
doi:10.1007/s00018-019-03059-8
40. Fuentealba RA, Udan M, Bell S, Wegorzewska I, Shao J, Diamond MI, Weihl CC, Baloh RH (2010) Interaction with polyglutamine aggregates reveals a Q/N-rich domain in TDP-43. *J Biol Chem* 285(34):26304–26314. doi:10.1074/jbc.M110.125039
41. Hasegawa M, Arai T, Nonaka T, Kametani F, Yoshida M, Hashizume Y, Beach TG, Buratti E, Baralle F, Morita M, Nakano I, Oda T, Tsuchiya K, Akiyama H (2008) Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Ann Neurol* 64(1):60–70.
doi:10.1002/ana.21425
42. Moisse K, Volkening K, Leystra-Lantz C, Welch I, Hill T, Strong MJ (2009) Divergent patterns of cytosolic TDP-43 and neuronal progranulin expression following axotomy: Implications for TDP-43 in the physiological response to neuronal injury. *Brain Res* 1249:202–211.
doi:https://doi.org/10.1016/j.brainres.2008.10.021

43. Nonaka T, Suzuki G, Tanaka Y, Kametani F, Hirai S, Okado H, Miyashita T, Saitoe M, Akiyama H, Masai H, Hasegawa M (2016) Phosphorylation of TAR DNA-binding Protein of 43 kDa (TDP-43) by Truncated Casein Kinase 1delta Triggers Mislocalization and Accumulation of TDP-43. *J Biol Chem* 291(11):5473–5483. doi:10.1074/jbc.M115.695379
44. Aulas A, Stabile S, Vande Velde C (2012) Endogenous TDP-43, but not FUS, contributes to stress granule assembly via G3BP. *Mol Neurodegeneration* 7:54–54. doi:10.1186/1750-1326-7-54
45. Sanchez II, Nguyen TB, England WE, Lim RG, Vu AQ, Miramontes R, Byrne LM, Markmiller S, Lau AL, Orellana I, Curtis MA, Faull RLM, Yeo GW, Fowler CD, Reidling JC, Wild EJ, Spitale RC, Thompson LM (2021) Huntington's disease mice and human brain tissue exhibit increased G3BP1 granules and TDP43 mislocalization. *J Clin Investig.* doi:10.1172/JCI140723
46. Labadorf A, Hoss AG, Lagomarsino V, Latourelle JC, Hadzi TC, Bregu J, MacDonald ME, Gusella JF, Chen J-F, Akbarian S, Weng Z, Myers RH (2015) RNA Sequence Analysis of Human Huntington Disease Brain Reveals an Extensive Increase in Inflammatory and Developmental Gene Expression. *PLoS ONE* 10(12):e0143563. doi:10.1371/journal.pone.0143563
47. Cuenca-Bono B, García-Molinero V, Pascual-García P, García-Oliver E, Llopis A, Rodríguez-Navarro S (2010) A novel link between Sus1 and the cytoplasmic mRNA decay machinery suggests a broad role in mRNA metabolism. *BMC Cell Biol* 11:19. doi:10.1186/1471-2121-11-19
48. Kim M, Choi Y, Kim H, Lee D (2019) SAGA DUBm-mediated surveillance regulates prompt export of stress-inducible transcripts for proteostasis. *Nat Commun* 10(1):2458. doi:10.1038/s41467-019-10350-6
49. Shiina N (2019) Liquid- and solid-like RNA granules form through specific scaffold proteins and combine into biphasic granules. *J Biol Chem* 294(10):3532–3548. doi:10.1074/jbc.RA118.005423
50. Kedersha N, Anderson P (2007) Mammalian Stress Granules and Processing Bodies. 431:61–81. 10.1016/S0076-6879(07)31005-7
51. Protter DSW, Parker R (2016) Principles and Properties of Stress Granules. *Trends Cell Biol* 26(9):668–679. doi:10.1016/j.tcb.2016.05.004
52. Furukawa Y, Kaneko K, Matsumoto G, Kurosawa M, Nukina N (2009) Cross-seeding fibrillation of Q/N-rich proteins offers new pathomechanism of polyglutamine diseases. *J Neurosci* 29(16):5153–5162. doi:10.1523/JNEUROSCI.0783-09.2009
53. Reineke LC, Neilson JR (2019) Differences between acute and chronic stress granules, and how these differences may impact function in human disease. *Biochem Pharmacol* 162:123–131. doi:10.1016/j.bcp.2018.10.009
54. Young JE, Gouw L, Propp S, Sopher BL, Taylor J, Lin A, Hermel E, Logvinova A, Chen SF, Chen S, Bredesen DE, Truant R, Ptacek LJ, Spada ARL, Ellerby LM (2007) Proteolytic Cleavage of Ataxin-7 by Caspase-7 Modulates Cellular Toxicity and Transcriptional Dysregulation. *J Biol Chem* 282(41):30150–30160. doi:10.1074/jbc.M705265200
55. Yvert G, Lindenberg KS, Devys D, Helmlinger D, Landwehrmeyer GB, Mandel JL (2001) SCA7 mouse models show selective stabilization of mutant ataxin-7 and similar cellular responses in different

- neuronal cell types. *Hum Mol Genet* 10(16):1679–1692. doi:10.1093/hmg/10.16.1679
56. Ström A-L, Jonasson J, Hart P, Brännström T, Forsgren L, Holmberg M (2002) Cloning and expression analysis of the murine homolog of the spinocerebellar ataxia type 7 (SCA7) gene. *Gene* 285(1–2):91–99. doi:10.1016/S0378-1119(02)00399-2
57. Edelstein A, Amodaj N, Hoover K, Vale R, Stuurman N (2010) Computer control of microscopes using µManager. *Curr Protoc Mol Biol* Chapter 14:Unit14.20-Unit14.20. doi:10.1002/0471142727.mb1420s92
58. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez J-Y, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A (2012) Fiji: an open-source platform for biological-image analysis. *Nat Methods* 9(7):676–682. doi:10.1038/nmeth.2019
59. Carpenter AE, Jones TR, Lamprecht MR, Clarke C, Kang IH, Friman O, Guertin DA, Chang JH, Lindquist RA, Moffat J, Golland P, Sabatini DM (2006) CellProfiler: image analysis software for identifying and quantifying cell phenotypes. *Genome Biol* 7:R100. doi:10.1186/gb-2006-7-10-r100

Figures

Figure 1

RNA binding protein TDP-43 co-localises with ATXN7 aggregates. a) Representative confocal images showing PC12 cells induced to express either ATXN7Q10 or ATXN7Q65, as well as non-induced controls. All are stained with ATXN7 in green and DRAQ5 (blue) used as a DNA marker. Contrast settings are the same for all images, within each stain. b) ATXN7Q10/Q65 expressing cells stained for ATXN7 (green) and TDP-43 (red), using DRAQ5 as a DNA marker. Arrows point to ATXN7 aggregates and co-localising TDP-43 staining. Contrast settings are the same for all images, within each stain. c) Representative profile plots showing the intensity values of ATXN7 and TDP-43 along a line drawn in b (merge). d) The percentages of sampled ATXN7 aggregates in cytoplasm and nucleus that contain a co-localising intensity peak of TDP-43 such as that displayed in c. e) Quantified fold change of total TDP-43 intensity per cell in ATXN7Q10/Q65 expressing or non-induced cells. n=3 for Q10, n=4 for Q65. Data are shown as mean ± SEM.

Figure 2

Phosphorylated TDP-43, but not total TDP-43, is increased in ATXN7Q65 expressing cells. a) Representative western blot showing ATXN7Q10/Q65 or non-induced PC12 cell extracts probed with α-p-TDP-43, α-TDP-43 and α-Tubulin. b) Quantified fold change of TDP-43 protein expression from blots

shown in a. c) Quantified fold change of p-TDP-43 protein expression from blots shown in a. (a-c: n=9) d) Representative western blot showing ATXN7Q10/Q65 or non-induced PC12 cell extracts probed with alternative p-TDP43 antibody, α-TDP-43 and α-Tubulin. e) Quantified fold change of p-TDP-43 protein expression from blots shown in d. (d-e: n=4). Data are shown as mean ± SEM, *: p<0.05.

Figure 3

TIA1 is sequestered into aggregates and is increased in ATXN7Q65 expressing cells. a) Representative confocal images of ATXN7Q10/Q65 expressing cells stained for ATXN7 (green) and TIA1 (red), using DRAQ5 as a DNA stain. Arrows point to ATXN7 aggregates and co-localising TIA1 staining. Contrast settings are the same for all images, within each stain. b) Quantified fold change of total TIA1 intensity per cell in ATXN7Q10/Q65 expressing or non-induced cells. c) Representative profile plots showing the intensity values of ATXN7, TIA1 and DRAQ5 along a line drawn in a (merge). d) The percentages of sampled ATXN7 aggregates in cytoplasm and nucleus that contain a co-localising intensity peak of TIA1 such as that displayed in c. (a-d: n=2 for Q10, n=3 for Q65) e) Representative widefield images of ATXN7Q10/Q65 expressing cells stained for ATXN7 (green) TIA1 (red), and G3BP1 (blue), using Hoechst as a DNA stain. Arrows point to ATXN7 aggregates and co-localising TIA1 staining. Contrast settings are the same for all images, within each stain. f) Quantified fold change of total TIA1 intensity per cell in ATXN7Q10/Q65 expressing or non-induced cells. g) Quantified fold change of total G3BP1 intensity per cell in ATXN7Q10/Q65 expressing or non-induced cells. h) Representative profile plots showing the intensity values of ATXN7, TIA1 and G3BP1 along a line drawn in e (ATXN7 panel). i) The percentages of sampled ATXN7 aggregates in cytoplasm and nucleus that contain a co-localising intensity peak of TIA1 such as that displayed in h, or G3BP1. (e-i: n=3 for Q10, n=4 for Q65). Data are shown as mean ± SEM, *: p<0.05.

Figure 4

The sequestration of TIA1 into ATXN7Q65 aggregates is not detergent resistant. a) Representative filter trap blot probed with α -ATXN7, α -TIA1, and α -G3BP1. Insoluble extracts from ATXN7Q65 expressing or non-induced PC12 cells were treated with SDS and DTT before filtration to capture aggregated material and immunoblotting. Contrast settings are the same for all images, within each stain. b) Quantified fold change of ATXN7 filter trap signal in ATXN7Q65 expressing cells treated with arsenite for 1 h compared to untreated induced cells. (n=7), Data are shown as mean $\pm$ SEM.

Figure 5

G3BP1 distribution exhibits more texture and a speckling behaviour in ATXN7Q65 expressing cells. a) Representative confocal images of ATXN7Q10/Q65 expressing or non-induced cells stained for ATXN7 (green) and G3BP1 (blue). b) Intensity heat maps of the respective G3BP1 stainings shown in a. c) Intensity surface plots of the respective G3BP1 stainings shown in a. d) Quantified fold change of the texture of the G3BP1 IF signal in ATXN7Q10/Q65 expressing or non-induced cells. e) Quantified fold change of the number of G3BP1 speckles in ATXN7Q10/Q65 expressing or non-induced cells. (n=4), data are shown as mean $\pm$ SEM, *: p<0.05, **: p<0.01.

Figure 6

G3BP1 granules are induced and altered in ATXN7Q65 cells. a) Representative widefield images of ATXN7Q10/Q65 expressing or non-induced PC12 cells treated with arsenite and stained for ATXN7 (green), TIA1 (red), and G3BP1 (blue), using Hoechst as a DNA stain. Thick arrows point to stress granules containing ATXN7, boxes surround stress granules that do not contain ATXN7, thin arrows point to ATXN7 aggregates containing TIA1. Contrast settings are the same across all images, within each stain. b) Quantified fold change of the number of G3BP1 granules, TIA1 granules, and stress granules (stained with both G3BP1 and TIA1) in induced PC12 cells compared to non-induced PC12 cells (n=4). c) Representative confocal images of G3BP1 granules in arsenite treated ATXN7Q65 induced PC12 cells compared to non-induced cells. d) Quantified eccentricity of G3BP1 granules in arsenite treated ATXN7Q65 induced PC12 cells compared to non-induced cells. (c-d: n=3). e) Representative confocal images of arsenite treated ATXN7Q10/Q65 expressing or non-induced PC12 cells showing co-localisation of ATXN7 with G3BP1 granules. Contrast settings are the same for all images, within each stain. f) Percentage of G3BP1 granules positive for ATXN7 in ATXN7Q10/Q65 expressing or non-induced cells. (e-f: n=3). Data are shown as mean $\pm$ SEM, *: p<0.05.

Figure 7

SCA7 patient fibroblasts show co-localisation of expanded ATXN7 with stress granules. a) Representative widefield images of human control fibroblasts (WT) compared to SCA7 patient fibroblasts (SCA7) stained for ATXN7 (green), TIA1 (red), and G3BP1 (blue). Contrast settings were optimised for untreated cells and arsenite treated cells separately. b) Quantified number of G3BP1, TIA1 and ATXN7 positive granules in arsenite treated fibroblasts. c) Representative profile plots showing the intensity values of G3BP1, TIA1, and ATXN7 in control fibroblasts, along a line drawn in a d) Representative profile plots showing the intensity values of G3BP1, TIA1, and ATXN7 in SCA7 fibroblasts, along a line drawn in a e) Quantified percentage of G3BP1 granules containing ATXN7 staining in human control fibroblasts and SCA7 patient fibroblasts (n=1, 51 WT cells and 54 SCA7 cells).

Figure 8

ATXN7Q65 expression does not affect the stress granule recovery rate after arsenite treatment. a) Schematic explaining the timeline of the experiment. b) Representative confocal images of ATXN7Q65 expressing or non-induced PC12 cells stained for G3BP1 after the treatment with and recovery from arsenite. c) Recovery rate of G3BP1 granules over time normalised to the number of granules at time point 0 (no recovery). d) Recovery of stress granules 30 minutes after removal of arsenite treatment in ATXN7Q65 expressing or non-induced cells, represented in fold change of non-induced cells. (b-d: n=4). Data are shown as mean $\pm$ SEM, ns = non significant.

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