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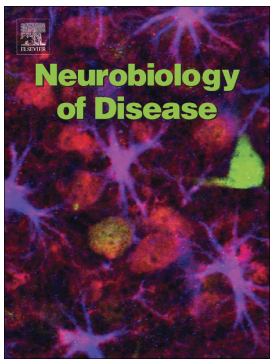

Pathogenesis of SCA3 and implications for other polyglutamine diseases

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Special Issue Review: Tandem Repeats

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Abstract
Tandem repeat diseases include the neurodegenerative disorders known as polyglutamine (polyQ) diseases, caused by *CAG* repeat expansions in the coding regions of the respective disease genes. The nine known polyQ disease include Huntington's disease (HD), dentatorubral–pallidoluysian atrophy (DRPLA), spinal bulbar muscular atrophy (SBMA), and six spinocerebellar ataxias (SCA1, SCA2, SCA3, SCA6, SCA7, and SCA17). The underlying disease mechanism in the polyQ diseases is thought principally to reflect dominant toxic properties of the disease proteins which, when harboring a polyQ expansion, differentially interact with protein partners and are prone to aggregate. Among the polyQ diseases, SCA3 is the most common SCA, and second to HD in prevalence worldwide. Here we summarize current understanding of SCA3 disease mechanisms within the broader context of the broader polyQ disease field. We emphasize properties of the disease protein, ATXN3, and new discoveries regarding three potential pathogenic mechanisms: 1) altered protein homeostasis; 2) DNA damage and dysfunctional DNA repair; and 3) nonneuronal contributions to disease. We conclude with an overview of the therapeutic implications of recent mechanistic insights.

Keywords
Ataxin-3; ATXN3; Spinocerebellar Ataxia; SCA3; Machado Joseph disease; MJD; Polyglutamine disease; Neurodegenerative disease; deubiquitinase

1. Introduction

Among the tandem repeat diseases, a subset of neurodegenerative diseases are caused by exonic CAG trinucleotide repeat expansions that are translated into abnormally long polyglutamine (polyQ) tracts in the disease proteins (Klockgether, Mariotti, and Paulson 2019). Currently, nine disorders comprise the so-called polyQ diseases, including Huntington's disease (HD), Dentatorubral–pallidoluysian atrophy (DRPLA), spinal bulbar muscular atrophy (SBMA), and six distinct spinocerebellar ataxias (SCAs) including SCA1, SCA2, SCA3, SCA6, SCA7, and SCA17 (Klockgether, Mariotti, and Paulson 2019). PolyQ diseases share several key features including that they: 1) are autosomal dominant disorders except SBMA, which is X-linked and sex-limited; (2) principally affect the central nervous system (CNS) although the peripheral nerves and muscle are variably affected; (3) usually present in adulthood and slowly progress over many years; (4) display neuropathological accumulation of disease protein aggregates, largely in neurons; and (5) are typically fatal disorders with no approved treatments to alter the disease course (Paulson et al. 2017).

Here we review what is known about the disease mechanisms underlying SCA3, the most common polyQ SCA, and relate this information to the broader polyQ field. We begin by introducing the disease protein, ATXN3, and summarizing the relevance of ATXN3 protein function and protein context to disease pathogenesis. We then highlight new discoveries regarding three potential pathogenic mechanisms in SCA3: 1) perturbations in cellular protein homeostasis; 2) disruption of DNA damage repair pathways; and 3) contributions of nonneuronal cells. We conclude with a discussion of how a thorough understanding of pathogenic mechanisms in SCA3 and related polyQ diseases is necessary to identify the most compelling therapeutic targets for these devastating, fatal, and currently untreatable disorders.

2. Genetic, Clinical, and Neuropathological features of SCA3

The neurodegenerative disorder now designated as SCA3 was first recognized in the 1970s in several families of Azorean descent as a heritable "ataxia-plus" disorder with overlapping clinical and pathological characteristics (Coutinho and Andrade 1978). Two of the identified families presenting with this dominantly inherited ataxia were descended from William Machado and Antone Joseph, leading to the disease designation Machado-Joseph Disease (MJD), a label still regionally used, particularly in Portuguese-speaking countries. In 1993, the genetic mutation causing SCA3 was mapped to chromosome 14q32.1 by two separate groups: one group studying the Azorean-linked MJD lineages and another European group which had designated the disease SCA3 after discovering that the genetic locus in families exhibiting similar symptoms to SCA1 and SCA2 was distinct from these two previously identified diseases (Takiyama et al. 1993; Gispert et al. 1993). In 1994, cloning of the SCA3 and MJD genetic loci identified the same polyQ-encoding CAG trinucleotide expansion, leading to convergence of these diseases into a single disorder, referred to here as SCA3 (Kawaguchi, Okamoto, Taniwaki, Aizawa, Inoue, Katayama, Kawakami, Nakamura, Nishimura, Akiguchi, and et al. 1994).

The CAG repeat expansion in SCA3 resides in exon 10 of the *ATXN3* gene (Haberhausen et al. 1995; Kawaguchi, Okamoto, Taniwaki, Aizawa, Inoue, Katayama, Kawakami, Nakamura, Nishimura, Akiguchi, Kimura, et al. 1994) (**Figure 1**). In healthy individuals, this CAG repeat ranges from 12 to 44, whereas in affected individuals the CAG repeat ranges from around 56 to 87 (Paulson 2007; Maciel et al. 1995; Maruyama et al. 1995; Durr et al. 1996). Individuals harboring CAG repeat lengths ranging from 45 to 55 present incomplete penetrance of SCA3 symptoms (Ashizawa, Öz, and Paulson 2018). As with other polyQ diseases, the CAG repeat length inversely correlates with age of disease onset and directly correlates with severity of disease (Maciel et al. 1995; Maruyama et al. 1995; Durr et al. 1996). SCA3 also exhibits "anticipation", a phenomenon shared across most polyQ diseases (Maciel et al. 1995; Maruyama et al. 1995). Anticipation refers to the tendency for expanded microsatellite repeats (e.g., the expanded *CAG* repeat in mutant *ATXN3*) to become increasingly long in subsequent generations (Durr et al. 1996), thereby typically causing more severe, earlier onset disease in offspring. This variability in CAG repeat length, in conjunction with (still poorly understood) genetic modifiers, contributes to the high variability in the clinical presentation of disease. Albeit rare, homozygous SCA3 patients have been reported, wherein an individual inherits two copies of the expanded mutant *ATXN3* gene that leads to more severe symptoms and earlier onset (Carvalho et al. 2008; Sobue et al. 1996). To our knowledge, no humans that are haploinsufficient or null for *ATXN3* have been reported.

SCA3 disease is believed to be the most common dominantly inherited form of ataxia, affecting approximately 1:50,000-100,000 people, and is second to HD in prevalence among the polyQ diseases (Durr 2010; Gardiner et al. 2019). While Portuguese SCA3 families have been shown to arise

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from tw 1). Still significant regional and ethnic differences in SCA3 prevalence have been reported, with the greatest prevalence reported in East Asian countries including China, Japan and Taiwan, as well as Portugal and Brazil (Buijsen et al. 2019). Symptom onset in SCA3 typically begins in adulthood between the third to fifth decade of life and progresses slowly with age (Rub et al. 2013). SCA3 patients exhibit a wide range of progressive motor impairments, including prominent cerebellar ataxia with abnormal gait, impaired balance, limb incoordination, dystonia, spasticity, dysarthria, dysphagia, and oculomotor abnormalities (Rub et al. 2013; Paulson et al. 2017). Parkinsonism with or without tremor also occurs in a subset of SCA3 patients (Park, Kim, and Jeon 2015). Though the severity and rate of decline varies across SCA3 patients, failure in brainstem-associated functions usually leads to death within 10-15 years of symptom onset (Diallo et al. 2018).

Progressive motor impairment in SCA3 results from neuronal dysfunction and neuronal cell loss in somatosensory and motor nuclei spanning the brainstem, cerebellum, midbrain, spinal cord, striatum, and thalamus (Rub et al. 2013). A prominent gross anatomical feature in SCA3 patients is enlargement of the fourth ventricle resulting from atrophy of neurons in the basilar pons and deep cerebellar nuclei, and loss of pontocerebellar fibers and spinocerebellar tracts (Durr et al. 1996; Rub et al. 2013). Post-mortem SCA3 disease brains exhibit significant degeneration of dopaminergic neurons of the substantia nigra and vestibular nuclei (Rub et al. 2004; Rub et al. 2013; Durr et al. 1996). SCA3 also leads to degeneration of motor neurons of the cranial nerve nuclei, red nucleus, subthalamic nucleus, globus pallidus, and some thalamic nuclei (Rub et al. 2003; Rub et al. 2013; Rüb et al. 2002; Rub et al. 2002). Spinal cord atrophy can occur in anterior horn, dorsal root ganglia, and dorsal nuclei (Fahl et al. 2015). Unlike in most other SCAs, olivary nuclei and the cerebellar cortex including Purkinje cells are relatively spared in SCA3 (Rub et al. 2013).

The cerebral cortex is affected in SCA3 patients but to a lesser extent than the brainstem and cerebellar nuclei (Yamada et al. 2001). By MRI, SCA3 patients exhibit reduced gray matter volume throughout temporal, frontal, parietal and insular areas (Lopes et al. 2013). Magnetic resonance spectroscopy (MRS) studies of SCA3 patients have identified metabolic abnormalities in white matter tracts beginning in premanifest SCA3 (Joers et al. 2018; Adanyeguh et al. 2015). Symptomatic SCA3 patients display mild cognitive impairments in executive function and memory, likely reflecting cerebral involvement in disease and/or disruptions to cerebellar-cerebral circuitry (Lopes et al. 2013). Sleep disorders are also common amongst patients, including REM sleep behavior disorder and restless leg disorder (Pedroso et al. 2011; Seshagiri et al. 2018). SCA3 pathology is not limited to the CNS: patients exhibit progressive peripheral neuropathy particularly in distal limbs leading to muscle atrophy and areflexia (Suga et al. 2014; Linnemann et al. 2016).

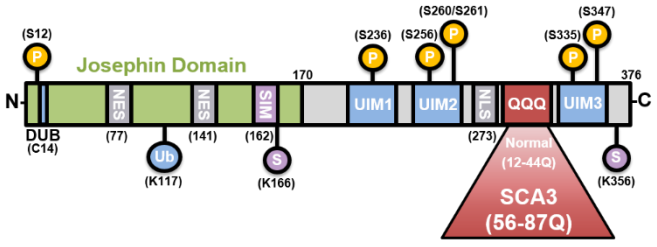
Accumulation of ubiquitinated protein aggregates or inclusions containing the mutant polyQ-expanded ATXN3 are found throughout vulnerable brain regions in SCA3 (Schmidt et al. 1998; Sittler et al. 2018; Paulson, Perez, et al. 1997). Neuronal nuclear inclusions (NNI) make up the majority of aggregates, though smaller neuronal cytoplasmic inclusions (NCIs) and distal axonal aggregates also occur (Hayashi, Kobayashi, and Furuta 2003; Seidel et al. 2010; Seidel et al. 2017). Similarly to aggregates in other polyQ diseases, ATXN3 NNI stain positively for many other proteins including ubiquitin, heat shock factor proteins, proteasomal subunits, transcription factors, autophagy-associated chaperones such as p62, other polyQ proteins, and non-expanded wildtype ATXN3 (Chai, Koppenhafer, Bonini, et al. 1999; Uchihara et al. 2001; Chai, Koppenhafer, Shoesmith, et al. 1999; Seidel et al. 2010; Seidel et al. 2017). The exact role that ATXN3 aggregation plays in SCA3 remains unclear, although it is generally accepted that the sequestration of critical protein quality control machinery into protein aggregates may contribute to neuronal stress. The same is likely true for other polyQ diseases (Paulson et al. 2017).

Scientists still do not fundamentally understand why only selective brain regions degenerate in SCA3 or other polyQ diseases, or why the *CAG* repeat length threshold for disease varies among the polyQ diseases. Clearly, understanding the normal functions of each polyQ protein will be critical to elucidating the cause of these distinct disease features in the polyQ disorders.

3. The SCA3 disease protein ATXN3

In polyQ diseases, most evidence points to aberrant actions of the disease protein as the principal driver of disease (reviewed in (Paulson et al. 2017)). The clinical and neuropathological differences *between* diseases likely reflects the unique protein context in which the polyQ expansion is embedded in each disease. Accordingly, to understand SCA3, we need to understand the SCA3 disease protein, ATXN3. ATXN3 (**Figure 1**) is a small, evolutionarily conserved protein that is ubiquitously expressed in the body and localized throughout the cell (Linhartová et al. 1999; Schmitt et al. 1997; Rodrigues et al. 2007; Paulson, Das, et al. 1997; Ichikawa et al. 2001). It is a deubiquitinase (DUB) implicated in protein quality control pathways (reviewed in (Costa and Paulson 2012)). DUBs are proteases that cleave ubiquitin from proteins, removing single ubiquitin modifications or cleaving polyubiquitin chains (Reyes-Turcu, Ventii, and Wilkinson 2009). ATXN3 binds polyubiquitinated proteins through three ubiquitin interaction motifs (UIMs) that flank the polyQ track, but the protein also contains additional ubiquitin binding sites in the catalytic domain known as the Josephin domain. ATXN3 binds the proteasome and preferentially cleaves ubiquitin chains that are four or more ubiquitins in length -- remarkably, the same chain length needed to target substrates efficiently to the proteasome (Thrower et al. 2000).

Figure 1. Schematic of ATXN3 protein. The N-terminal Josephin domain contains the deubiquitinase (DUB) catalytic site (C14), as well as nuclear export sites (NES) and a small ubiquitin-like modifier [SUMO]-interacting motif (SIM). The C-terminal tail contains two or three ubiquitin-interacting motifs (UIMs), a putative nuclear localization signal (NLS) and the variable polyQ repeat. Repeat lengths of normal and pathogenic polyQ are shown. ATXN3 can be post-translationally modified by ubiquitin (Ub) at K117, sumoylation (S) at K166 and K356, and phosphorylation at S12, S236, S256, S260, S261, S335, and S347.



The preferential activity of ATXN3 toward longer poly-ubiquitin chains coupled with its known interaction with several ubiquitin ligases suggest that ATXN3 (Scaglione et al. 2011; Durcan et al. 2011; Matsumoto et al. 2004) edits ubiquitin chain length and composition on substrates destined for proteasomal degradation. The function of ATXN3, however, likely extends beyond the ubiquitin proteasome system (UPS) to other processes including macroautophagy, transcriptional regulation, cytoskeletal organization, and DNA damage repair (Doss-Pepe et al. 2003; Costa and Paulson 2012; Li et al. 2002; Araujo et al. 2011; Evert et al. 2003; Rodrigues et al. 2010; Gao et al. 2019; Gao et al. 2015; Burnett, Li, and Pittman 2003).

ATXN3 possesses several domains that contribute to its function (**Figure 1**). The structured globular amino-terminus contains the highly conserved Josephin domain responsible for catalytic cleavage of ubiquitin chains. The flexible C-terminal tail contains three ubiquitin interacting motifs (UIMs) that facilitate ATXN3 binding to poly-ubiquitinated chains (Costa and

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Paulso [illegible] oforms have been identified, but most studies have focused on two full-length isoforms: one expressing all three UIMs (3UIM ATXN3) and one lacking the third UIM (2UIM ATXN3) (Harris et al. 2010). 3UIM ATXN3 appears to be the major isoform expressed in brain (Harris et al. 2010). The presence or absence of the third UIM may alter the specificity of ATXN3 towards poly-ubiquitin chains and substrates (Weishaupl et al. 2019).

Knocking out ATXN3 *in vitro* or in mice leads to increased levels of total ubiquitinated proteins, suggesting ATXN3 is a promiscuous DUB (Schmitt et al. 2007). Indeed, studies have identified over 100 substrates or binding partners of ATXN3 (Paulson et al. 2017). Through the editing of ubiquitin chains, ATXN3 can alter a substrate's stability or activity in various ways depending on the substrate and the length or type of ubiquitin chain (Paulson et al. 2017). *In vitro* studies have shown that ATXN3 preferentially edits chains of at least four ubiquitin moieties (Winborn et al. 2008). A tetraubiquitin chain is also the minimum length required for poly-ubiquitinated substrates to be recognized by the 26S proteasome (Thrower et al. 2000). By binding the minimum length poly-ubiquitin chain on substrates, ATXN3 may prevent complete removal of the ubiquitin chain by other DUBs, and thus facilitate its recognition by the 26S proteasome (Li et al. 2015). ATXN3 DUB activity can also prevent or diminish substrate delivery to the proteasome, such as with the autophagy-related substrate Beclin1 and DNA damage repair and cell cycle proteins, Chk1 and p53 (Tu et al. 2017; Liu et al. 2016; Ashkenazi et al. 2017).

The presence of a polyQ expansion does not prevent mutant ATXN3 from binding poly-ubiquitin chains or deter its enzymatic activity *in vitro* (Winborn et al. 2008; Burnett, Li, and Pittman 2003). However, the polyQ expansion may alter its substrate specificity or protein-protein interactions in subtle ways that are not yet fully understood. Indeed, total levels of poly-ubiquitinated proteins are increased in the SCA3 brain, which may support some loss-of-function contributions to disease (Sittler et al. 2018). Broad disruptions to UPS as a result of sequestration of UPS regulators into mutant ATXN3 aggregates also likely contribute to the accumulation of poly-ubiquitinated proteins in SCA3 (Chai, Koppenhafer, Shoesmith, et al. 1999).

ATXN3 localizes both to the cytoplasm and nucleus to a varying degree depending on the cell type, and its transport across the nuclear membrane appears to be highly dynamic. Full-length ATXN3 possesses two nuclear export sequences (NES) and a putative nuclear localization sequence (NLS) that facilitate active transport across the nuclear membrane (Antony et al. 2009; Sowa et al. 2018) (**Figure 1**). In many cell types ATXN3 predominantly localizes to the cytoplasm under basal conditions (Antony et al. 2009), but cellular stressors such as oxidative stress or heat shock trigger rapid nuclear localization of ATXN3 (Reina, Zhong, and Pittman 2010). As noted earlier, the abnormal concentration and aggregation of mutant ATXN3 in neuronal nuclei is a defining feature of SCA3 neuropathology (Rub et al. 2013; Paulson, Perez, et al. 1997). Interestingly, preventing nuclear localization of mutant ATXN3 mitigates many disease features, whereas forcing mutant ATXN3 into the nucleus enhances disease in SCA3 mice (Bichelmeier et al. 2007).

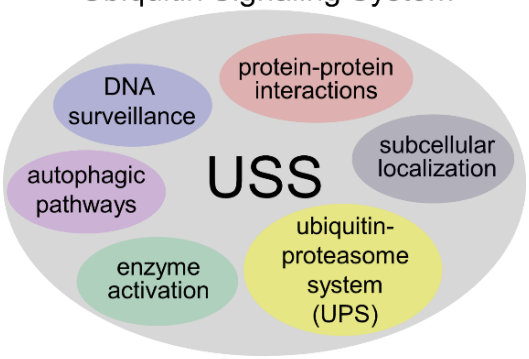
ATXN3 is subjected to numerous post-translational modifications (PTMs), including ubiquitination at K117, SUMOylation, and phosphorylation (**Figure 1**). All three of these PTMs alter ATXN3 behavior and function. Ubiquitination at K117 potentiates the DUB activity of ATXN3 (Todi et al. 2010; Todi et al. 2009), and phosphorylation at specific sites can alter nucleocytoplasmic localization of ATXN3 (Mueller et al. 2009). While ATXN3 can bind SUMO and be SUMOylated (Zhou et al. 2013; Almeida et al. 2015), it is not yet clear how SUMO binding or SUMOylation alters ATXN3 function or clearance. In one study, mutating a SUMO binding site in ATXN3 accelerated the degradation of expanded but not normal ATXN3 (Almeida et al. 2015), suggesting that SUMO effects could be particularly germane to the mutant form of the disease protein. More work is needed to assess how these various posttranslational modifications might modulate disease pathogenesis.

3.1 Role of altered protein homeostasis

Perturbations in protein homeostasis represent a recurring theme in the polyQ diseases including SCA3 (Paulson et al. 2017; Klockgether, Mariotti, and Paulson 2019). The three major arms of protein quality control (PQC) --the UPS, molecular chaperones, and autophagy -- have all been implicated in SCA3 and other polyQ diseases. In these diseases, a consequence of continually producing aggregate-prone proteins (e.g. polyglutamine expanded ATXN3) is that PQC pathways may fail to keep pace. Moreover, the aggregation of ATXN3 and other polyQ disease proteins can sequester components of PQC pathways, exacerbating the problem. Among polyQ diseases, this issue is compounded in the case of SCA3 since the protein, a DUB, itself directly participates in PQC. In this section we review the role of ATXN3 in PQC pathways and the evidence for impairment of such pathways in disease.

As mentioned earlier, ATXN3 is a quality control DUB most closely linked to the UPS. PolyQ-expanded ATXN3 continues to bind and cleaves polyQ chains in simple cellular and in vitro assays. Under more complex physiological conditions, however, relatively little is known about how polyQ expansion affects the capacity of ATXN3 to regulate ubiquitination, the type of chain linkages it can cleave, and the efficiency of proteasomal delivery of substrates. Ubiquitin and proteasomal subunits are known to be sequestered in intraneuronal aggregates in SCA3 disease brain and mouse models (Seidel et al. 2010; Chai, Koppenhafer, Shoesmith, et al. 1999; Schmidt et al. 2002). While such sequestration may constrict neuronal UPS capacity, this has not been firmly established. But robust impairment of UPS activity may not be required to have a deleterious effect in SCA3. We suggest that even rather modest effects on UPS capacity could have profound indirect effects on other aspects of ubiquitin signaling within neurons or other cell types. **Figure 2** illustrates these other components of ubiquitin signaling, to which greater attention should be paid in studies of SCA3 and other polyQ disorders.

Figure 2. Components of the Ubiquitin Signaling System. Ubiquitin conjugation to proteins serves diverse roles beyond the ubiquitin proteasome system (UPS), including roles in DNA repair, autophagy, subcellular localization and trafficking, and alterations in protein interactions. As a DUB, ATXN3 has been most closely associated with the UPS but increasingly is linked to other ubiquitin-dependent pathways shown here. Accordingly, in studies of SCA3 and other polyQ diseases, more attention should be paid to broader impairments in the ubiquitin signaling system beyond the UPS. Perturbations in ubiquitin homeostasis in one arm (e.g. the UPS) could have important, indirect effects on other arms of the ubiquitin signaling system (USS).



In various neurodegenerative diseases, an overwhelming of the UPS by accumulation of misfolded proteins can trigger aggresome formation, a cellular pathway that serves to consolidate proteotoxic aggregates (Kopito 2000). Aggresomes are dynamic perinuclear structures regulated by elements of the UPS machinery, including E3 ligases, proteasomal subunits and K63-linked poly-ubiquitin chains, and the autophagy-linked protein p62 (Olzmann, Li, and Chin 2008). Formation of aggresomes also relies on microtubule-dependent transport of aggregated proteins. Non-pathogenic ATXN3 exhibits a preference towards K63 ubiquitin linkages (Winborn et al. 2008), and directly interacts with many cytoskeletal proteins, proteasomal regulators, and autophagic proteins involved in aggresome formation, including tubulin, dynein, HDAC6, Parkin, CHIP, and p62 (Bonanomi et al. 2014; Mazzucchelli et al. 2009; Scaglione et al. 2011; Zhou et al. 2014). ATXN3 also localizes to, and regulates formation of, aggresomes containing misfolded disease proteins implicated in neurodegenerative disease, including mutant SOD1 and CFTR (Wang, Ying, and Wang 2012; Burnett and Pittman 2005). The ability of ATXN3 to mediate aggresome formation is ablated by mutating catalytic sites or removing UIMs, suggesting that ATXN3's DUB-dependent roles are critical to aggresome formation (Burnett and Pittman 2005).

For over two decades, studies have established a link between SCA3 and a variety of molecular chaperones (Chan et al. 2000; Warrick et al. 1999; Chai, Koppenhafer, Bonini, et al. 1999; Vos et al. 2010; Ito et al. 2016; Seidel et al. 2012). Numerous chaperones, when overexpressed, can mitigate disease features in SCA3 model systems either by improving the "handling" of mutant ATXN3 or by boosting overall protein homeostasis, or both. Earlier studies established a general role for Hsp40 in Hsp70 chaperones, with more recent studies identifying specific brain-expressed chaperones as particularly relevant to SCA3 (e.g. HSPB7, HSPB8-BAG3, and DNAJC8). While many studies support a role for a classic ATP-dependent action of molecular chaperones to re-fold or disaggregate mutant ATXN3, some studies support links between the implicated chaperones and the autophagy-lysosomal system (Pavel et al. 2016). Just as various components of the ubiquitin signaling system may affect one another, the three major arms of PQC are closely interlinked and can profoundly affect one another.

Links between SCA3 and autophagy have recently emerged. In SCA3 disease brain, for example, levels of key autophagy proteins are dysregulated (Sittler et al. 2018). Boosting cholesterol pathways in the brain can improve autophagy and mitigate disease features in a mouse model of SCA3 (Nobrega et al. 2019). Similarly, the protein translation inhibitor Cordycepin and the calpain inhibitor calpeptin mitigate disease features in SCA3 disease models, presumably through a mechanism associated with enhanced autophagy (Marcelo et al. 2019; Watchon et al. 2017)

Intriguingly, ATXN3 appears to be directly tied to autophagy: Several studies have identified regulatory roles for wildtype ATXN3 in macroautophagy. In particular, ATXN3 binds to and regulates levels of Beclin1, a key protein within the autophagy pathway involved in nucleation of autophagosomes (Ashkenazi et al. 2017). The ATXN3-Beclin1 interaction stabilize Beclin1, preventing its degradation by the proteasome (Ashkenazi et al. 2017). PolyQ-expanded mutant ATXN3 similarly binds to Beclin1, but appears to facilitate its delivery and degradation by the proteasome, resulting in reduced Beclin 1 levels (Ashkenazi et al. 2017). Cellular and animal models of SCA3 exhibit altered expression of key autophagy proteins that may be caused in part by polyQ-dependent destabilization of Beclin1. SCA3 patient-derived fibroblasts exhibit reduced levels of Beclin1, decreased autophagic flux, and impaired formation of autophagosomes (Onofre et al. 2016). Beclin1 levels are significantly reduced in SCA3 patient brains, while other key-autophagy proteins, including ATG-12, LAMP-2, Rab7, Rab1A, and LC3, are significantly increased (Sittler et al. 2018). Autophagy proteins like p62 and LC3 also localize to ATXN3 aggregates in vulnerable brain regions in SCA3 patients (Sittler et al. 2018). Thus, perturbations of PQC in SCA3 may well be the culminating effect of accumulation of mutant ATXN3 aggregates, sequestration of key UPS and autophagic regulators, and destabilization of Beclin1.

3.2 DNA Damage & Repair Pathways in SCA3

DNA is continually exposed to exogenous and endogenous stressors leading to DNA breaks (Madabhushi, Pan, and Tsai 2014). Damaged or improperly repaired DNA can become oncogenic, activate pro-apoptotic pathways, or induce cellular senescence (Jackson and Bartek 2009; Iyama and Wilson 2013). Cells have developed complex DNA damage signaling and repair mechanisms, collectively called the DNA damage response (DDR), to deal with a variety of DNA insults (Jackson and Bartek 2009; Iyama and Wilson 2013). Activation of DDR pathways can trigger widespread transcriptional changes (Gregersen and Svejstrup 2018) and activate protein quality control pathways including the UPS and macroautophagy (Xie and Jarosz 2018). Conversely, inhibition of macroautophagy can directly inhibit DNA damage repair processes (Wang, Zhu, and Zhao 2017).

Increased accumulation of damaged DNA in neurons is characteristic of normal aging, but is also a key feature of many neurodegenerative diseases including in Alzheimer's Disease, Parkinson's Disease, and HD (Shanbhag et al. 2019; Milanese et al. 2018; Ferlazzo et al. 2014; Amirifar et

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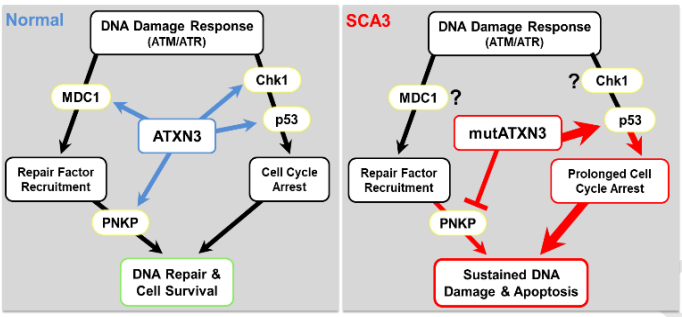
al. 201 genes encoding key DNA damage repair proteins. For instance, mutations in the *ataxia telangiectasia mutated (ATM)* gene, a global regulator of DNA damage signaling and cell cycle arrest, causes Ataxia Telangiectasia, a recessive ataxia affecting both CNS and peripheral tissues (Rothblum-Oviatt et al. 2016; Shiloh and Ziv 2013). Several recessive ataxias exhibiting CNS-limited degeneration are caused by mutations in DNA single-strand break (SSB) repair genes, suggesting the CNS may be exceptionally vulnerable to disruptions to SSB repair (Caldecott 2003; Massey and Jones 2018).

Mounting evidence suggests that compromised DNA repair also contributes to SCA3 disease pathogenesis. Normal and polyQ-expanded ATXN3 are both rapidly recruited to DNA damage foci in cells exposed to genotoxic stress, though it remains unclear what mediates ATXN3 nuclear import and translocation to damaged chromatin (Pfeiffer et al. 2017). Enhanced accumulation of DNA damage has been observed in SCA3 patient post-mortem tissue (Chatterjee et al. 2015) and transgenic animal models of SCA3 (Kazachkova et al. 2013). Expression of polyQ-expanded mutant ATXN3 also decreases DNA repair efficiency and enhances vulnerability of cells following irradiation or exposure to genotoxins (Liu et al. 2016). And recent GWAS studies have identified DNA mismatch repair genes as modifiers of age-of-onset in polyQ diseases collectively, although the particular relevance of mismatch repair pathways to SCA3 is limited by the relatively small SCA3 population size included in this study (Bettencourt et al. 2016). **Figure 3** highlights what is currently known about the normal roles for ATXN3 in mediating the DNA damage response (DDR) and how DNA repair may be impaired by polyQ-expanded mutant ATXN3.

Figure 3. Normal and pathogenic roles of ATXN3 in the DNA damage response (DDR). The DDR is a series of complex interconnected pathways that function to detect, signal, and repair DNA damage. DDR is initiated by phosphorylation of ATM or ATR apical kinases. Activated ATM/ATR phosphorylate mediator proteins (i.e. MDC1) that remodel damaged chromatin leading to recruitment of DNA repair factors (i.e. PNKP) that process and repair DNA. Cell cycle regulators (i.e. Chk1 and p53) are also activated by ATM/ATR to stall cell cycle progression during DNA repair. (Left) Normal ATXN3 regulates the kinetics of early and late events in the DDR process. As a deubiquitinase, ATXN3 stabilizes MDC1, Chk1, and p53 through the removal of poly-ubiquitin chains, and promotes PNKP phosphatase activity through an unclear mechanism, to promote DNA repair and cell survival. (Right) Conversely, polyglutamine-expanded mutant ATXN3 (mutATXN3) inhibits PNKP-dependent DNA repair and prolongs p53-signaling through enhanced p53-binding and stabilization. Unrepaired DNA damage and/or sustained p53-activation by mutATXN3 leads to increased activation of pro-apoptotic pathways and cell vulnerability to DNA damage. (MDC1=mediator of DNA damage checkpoint protein 1; Chk1=checkpoint protein 1; p53=tumor protein 53; PNKP=polynucleotide kinase-phosphatase; ATM=ataxia telangiectasia mutated; ATR=ataxia telangiectasia and Rad3-related protein).



The existing literature suggests versatile DUB-dependent roles for ATXN3 in fine-tuning the temporal and spatial dynamics of key DDR proteins at DNA damage foci. DDR pathways are activated by two apical DNA kinases, ATM or ATR (ATM and rad3-related) kinase (Marechal and Zou 2013; Smith et al. 2010). Several studies demonstrate that ATXN3 stabilizes early targets of ATM- and ATR-coupled DDR pathways to promote DNA repair and checkpoint signaling. MDC1 (mediator of DNA damage checkpoint 1) is one of the earliest proteins activated by ATM (Lou et al. 2006). Activated MDC1 localizes to damaged DNA where it recruits E3 ligases that ubiquitylate surrounding chromatin, a necessary step for recruitment of scaffolding and repair factors like 53BP1 and BRCA1 (Mailand et al. 2007). MDC1 stability and dwell-time at chromatin is tightly regulated by the antagonistic enzymatic activities of ATXN3 and E3 ubiquitin ligase RNF4 (Pfeiffer et al. 2017). Interestingly, the recruitment of both RNF4 [a known SUMO-targeted ubiquitin ligase (STUbL)] and ATXN3 to sites of DNA damage can be abolished through inhibition of DDR-coupled SUMOylation, suggesting ATXN3 may act as a SUMO-targeted DUB within the DDR pathway (Pfeiffer et al. 2017). As a DUB, ATXN3 stabilizes the ATR downstream targets Chk1 (Checkpoint Kinase 1) and p53 (tumor protein 53), both of which function to delay cell cycle progression and promote DNA repair (Liu et al. 2016; Tu et al. 2017). Chk1 and p53 can also activate pro-apoptotic pathways in the presence of sustained unrepaired DNA and replicative stress (Fridman and Lowe 2003; Matsuura et al. 2008). In these studies, depleting endogenous ATXN3 led to increased levels of the poly-ubiquitinated DDR substrates (i.e. MDC1, Chk1, or p53), compromised DNA repair and checkpoint signaling, and enhanced cellular vulnerability to genotoxic stress (Chatterjee et al. 2015; Gao et al. 2015; Liu et al. 2016). Interestingly, over-expressing non-expanded ATXN3 *in vitro* only partially rescued or actually enhanced cellular vulnerability to genotoxins (Pfeiffer et al. 2017; Tu et al. 2017), suggesting that ATXN3 levels and activity are tightly regulated within the DDR.

Clearly, loss of ATXN3 can impair multiple DNA repair processes. But how the polyQ expansion alters ATXN3 DUB-dependent roles in DDR is less clear. Mutant ATXN3 aggregation has been shown to sequester wildtype ATXN3 (Uchihara et al. 2001), potentially depleting cellular stores of ATXN3 and leading to decreased ATXN3 interactions with and destabilization of DDR substrates. On the other hand, enhanced nuclear localization (which is characteristic of mutant ATXN3) could lead to inappropriate or prolonged mutant ATXN3 stabilization of DDR proteins. Some evidence supports the latter hypothesis. In zebrafish and mouse models of SCA3, mutant ATXN3 exhibited an increased affinity for p53, leading to prolonged activation of p53 and p53 target genes and enhanced p53-dependent neurodegeneration (Liu et al. 2016). PolyQ-expanded mutant ATXN3 appeared to have equivalent Chk1 DUB capacity in HEK293 cells (Tu et al. 2017), but whether this is true in neuronal cells or *in vivo* models of SCA3 remains unclear. In addition, no studies have investigated whether polyQ-expanded mutant ATXN3 exhibits altered interactions with the DUB substrate MDC1.

Normal and mutant ATXN3 also interact with PNKP (polynucleotide kinase 3'-phosphatase), an enzyme directly involved in processing and repair of DNA damage. Two studies in 2015 showed that ATXN3 binds to and enhances PNKP 3'-phosphatase activity, promoting DNA repair (Chatterjee et al. 2015; Gao et al. 2015). However, how ATXN3 enhances PNKP activity was not clear from these studies. Conversely, polyQ-expanded mutant ATXN3 inhibited PNKP activity, possibly as a result of PNKP sequestration into ATXN3 aggregates (Chatterjee et al. 2015). Knockdown of normal ATXN3 or PNKP, or expression of mutant ATXN3 increased the accumulation of DNA damage and persistent activation of the ATM/p53-dependent DNA repair pathway, suggesting ATXN3 may be a key regulator of PNKP-dependent DNA repair (Chatterjee et al. 2015; Gao et al. 2015). A more recent study demonstrated that ATXN3 and PNKP also stably associate with transcription-coupled DNA repair (TCR) complexes composed of POLR2A, CBP, and the polyQ-containing HTT protein (Gao et al. 2019). In this study, expression of polyQ-expanded HTT or depletion of endogenous ATXN3 led to impaired

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TCR. K quired, however, to elucidate the normal and pathogenic roles of ATXN3 in TCR.

To summarize, in this section we have highlighted four recently described ATXN3 DUB substrates or interacting proteins whose functions span DNA repair factor recruitment, cell cycle arrest, and DNA repair (**Figure 3**). Based on recent studies, we suggest that wildtype ATXN3 stabilizes, or regulates, proteins at various step of the DDR process to promote efficient repair and cell survival. However, as evidenced by widespread changes in protein ubiquitylation in the *Atxn3* knockout mouse brain (Schmitt et al. 2007), ATXN3 likely has more, as yet unidentified, DDR substrates. Future studies are also needed to discern how wildtype ATXN3 recruitment to, and removal from, DNA damage foci is regulated. Insights into normal ATXN3 kinetics within the DDR could shed light on how mutant ATXN3 directly or indirectly disrupts DNA repair. Future studies aimed at understanding the relevance of loss-of-function or gain-of-function mutant ATXN3 contributions to deficient DNA repair may also inform gene-silencing therapies for SCA3. Finally, the relevance of different DDR pathways varies across proliferating, non-proliferative dividing cells, and long-lived non-dividing cells (Massey and Jones 2018), all of which are present in the CNS. Improved understanding of cell type-specific differences in DNA repair pathways in the CNS could point to regional and cell-type specific vulnerabilities to neurodegeneration in SCA3 and related neurodegenerative diseases.

3.3 Non-neuronal contributions to SCA pathogenesis

Due to the selective degeneration of neuronal populations, most research in polyQ disorders has taken a neuron-centric point of view. It is important to note, however, that glial cells, once only considered secondary supporting cells, are now recognized as vital components of the CNS that contribute greatly to neuronal health. All three types of glia cells in the brain -- astrocytes, microglia and oligodendrocytes -- have recently been implicated in SCA3 disease pathology and more broadly in polyQ diseases. All three cell types may interact with neurons to abrogate disease pathogenesis.

Astrocytes:

Astrocytes help regulate the transmission of electrical impulses in the CNS by assisting with ion homeostasis at neuronal synapses (Liddelow and Barres 2017). They also function to protect cells (both neurons and other glia) from stress and reduce the spread of inflammatory cells to areas of disease damage (Sofroniew 2009). When CNS tissue is damaged, astrocytes are activated. Astrocyte activation can be visualized by increased glial fibrillary acid protein (GFAP) immunostaining, which occurs in regions of disease pathology in SCA3 (Scherzed et al. 2012; Rub et al. 2002; Rüb et al. 2002). Similarly, multiple SCA3 mouse models demonstrate increased GFAP-positive astrocytes in brainstem and cerebellar white matter (Silva-Fernandes et al. 2010; Switonski et al. 2015; Cemal et al. 2002). Whether astrocyte activation in SCA3 is cell autonomous or an indirect byproduct of neuronal damage/stress remains to be tested. Studies in other polyQ animal models expressing mutant proteins selectively in astrocytes recapitulate some aspects of disease (Yang et al. 2017; Bradford et al. 2009), and conditional knockdown of mutant *HTT* in astrocytes conferred partial therapeutic benefit in a HD mouse model (Wood et al. 2019). These results imply a possible synergistic toxicity of mutant polyQ proteins in neurons and astrocytes that remains to be tested in SCA3 models.

Microglia:

Microglia are the immune cells of the CNS that, when activated by insult or injury, transform into cells that phagocytose dead or dying cells and cellular debris and release proinflammatory signals such as cytokines, proteases and oxygen radicals to mitigate damage (Hickman et al. 2018). While reactive microgliosis is observed in SCA3 patients (Evert et al. 2001; Rub et al. 2002; Rüb et al. 2002), follow-up studies exploring the basis of microglial activation in SCA3 are essentially lacking. One study of serum cytokine levels found eotaxin to be increased in asymptomatic SCA3 patients relative to symptomatic SCA3 patients and normal controls (da Silva Carvalho et al. 2016). Eotaxin is secreted by activated astrocytes, whereas microglia predominantly express its receptor (Parajuli et al. 2015). High eotaxin expression can lead to exacerbated ROS and putative cell death (Parajuli et al. 2015). Early increased expression of eotaxin before the onset of symptoms may suggest glial involvement in early stages of disease. Understanding how eotaxin levels are regulated in SCA3 remains to be determined.

In another polyQ disease, SCA1, studies in mouse models have demonstrated that glial pathology (microglia and astrocytes) correlates with disease onset and severity and is induced by neuronal dysfunction (Cvetanovic et al. 2015). Interestingly, reducing microglia early in the SCA1 disease process improved motor deficits in SCA1 mice (Qu et al. 2017). In HD, activated microglia are associated with peripheral cytokine levels in asymptomatic HD patients (Politis et al. 2015) and mutant *HTT* expressed only in mouse microglia is sufficient to trigger neurodegeneration (Crotti et al. 2014). Assessment of early microglial dysfunction in SCA3 and other polyQ diseases may provide insight into the early pathogenic mechanisms of disease prior to symptom onset.

Oligodendrocytes:

As myelin-producing cells that ensheath and support neuronal axons, oligodendrocytes constitute the main component of white matter tracks in the CNS. They play integral roles in myelin-rich white matter which is vulnerable in SCA3 (Rüb, Brunt, and Deller 2008; Kang et al. 2014). Based on brain imaging, white matter changes in the brainstem appear to be an early disease feature in human SCA3 disease (Lukas et al. 2006; Rezende et al. 2018; Kang et al. 2014; D'Abreu et al. 2009; Guimaraes et al. 2013).

Data in SCA3 mouse models support transcriptional dysregulation in oligodendrocytes, with the top identified disrupted pathways being myelination, axon ensheathment and cholesterol biogenesis (Ramani et al. 2017; Toonen et al. 2018). High cholesterol levels are required for myelin membrane growth (Saher et al. 2005), as well as axon growth and synapse remodeling in the adult brain (Dietschy 2009). Because of the blood-brain barrier, most cholesterol in the CNS must be produced via *de novo* synthesis (Orth and Bellosta 2012). CNS neurons can produce enough cholesterol to survive, but proper synapse formation demands additional cholesterol generated by surrounding oligodendrocytes and astrocytes (Mauch et al. 2001; Martin, Pfrieger, and Dotti 2014). A recent study that restored neuronally synthesized cholesterol in the SCA3 mouse brain by overexpressing the rate-limiting enzyme for brain cholesterol efflux was able to rescue many aspects of SCA3 animal model pathology and behavior (Nobrega et al. 2019). Previous research links perturbations in cholesterol homeostasis to many neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, HD and SCA3 (Vance 2012). Analysis of HD mouse and human disease brain tissue revealed significantly decreased cholesterol biosynthesis due to the downregulation of cholesterol biosynthesis genes in cells expressing mutant HTT (Huang et al. 2015). A more recent study showed that removing mutant HTT protein from oligodendrocytes partially rescued aspects of HD pathology (Ferrari Bardile et al. 2019). Together, these noted alterations in myelination and cholesterol biosynthesis strongly support a significant role for early oligodendrocyte dysfunction in SCA3 and possibly other polyQ diseases.

Clearly, nonneuronal cells of the CNS contribute to pathogenesis of SCA3 and other polyQ diseases. Future studies should seek to tease apart the relationship of these cell types to disease progression rather than simply capture a static snapshot of disease in postmortem samples. Disease-specific

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stem c isease processes. Currently there are multiple human SCA3 induced pluripotent stem cells that can be used to study disease pathogenesis and test potential therapies (Hansen et al. 2016; Koch et al. 2011; Ou et al. 2016; Ouyang et al. 2018; Ritthaphai et al. 2018; Chuang et al. 2019). Our group recently reported the first NIH-approved human SCA3 embryonic stem cell line which recapitulates well-established SCA3 molecular phenotypes both at the stem cell and differentiated states (Moore et al. 2019). An important gap in SCA3 model systems is the lack of conditional mouse models in which scientists can express or silence mutant ATXN3 in neurons or various nonneuronal cell types in order to define cell-autonomous versus non-cell autonomous contributions to disease.

4. Recurring themes in polyglutamine diseases

Understandably, the above sections on perturbations in protein homeostasis, DNA damage and repair pathways, and functions of nonneuronal cells in disease pathogenesis focus on SCA3 and its disease protein. But the observations are not unique to this polyQ disorder. Rather, they represent recurring themes across essentially the entire polyQ disease spectrum. For example, the widespread presence of mislocalized and aggregated disease protein in essentially all polyQ diseases (possibly except SCA6) underscores the likely involvement of perturbed protein homeostasis in the entire disease class, although the precise details will differ based on the specific disease protein, its normal functions, and its normal interacting partners. Evidence increasingly suggests there are failures in DNA integrity and, more generally, nuclear integrity in polyQ diseases. Studies of DRPLA, for example, elegantly link failures in nuclear integrity to problems in autophagy (Baron et al. 2017), and genetic linkage and studies in model systems have linked DNA repair pathways to HD and several polyQ ataxias (reviewed in (Maiuri et al. 2019; Jones, Houlden, and Tabrizi 2017)). The role of non-neuronal cells in disease pathogenesis is well illustrated by the central importance of Bergmann glia to the process of cerebellar degeneration in SCA7 (Furrer et al. 2011), and activation of microglia may be a common feature in polyQ diseases, as shown in mouse models of SCA1 and HD (Cvetanovic et al. 2015; Crotti et al. 2014). These shared elements of pathogenesis across the spectrum of polyQ diseases raise the intriguing prospect of disease-modifying therapy that is applicable to more than one such disease. At the same time, we must acknowledge that, in each disease, the polyQ expansion occurs in vastly different proteins with entirely different functions and few shared interacting partners. This disease-specific protein context is the principal reason why each disorder is clinically distinct with its own characteristic pattern of neurodegeneration. As discussed below, it is also the reason why the search for disease-specific therapies must continue at the same time that broadly applicable therapies are sought.

5. Therapeutic implications

Although the genetic cause of SCA3 disease has been known for decades, SCA3 is still a fatal, untreatable disease. Strides are being made towards better disease understanding and potential treatment (Ashizawa, Öz, and Paulson 2018; Duarte-Silva and Maciel 2018; McLoughlin et al. 2018). Nevertheless, much remains unknown about how the *CAG* expansion in the *ATXN3* gene causes brain dysfunction and cell death, manifesting in a characteristic clinical syndrome. As discussed here and in other reviews (Ashizawa, Öz, and Paulson 2018; Matos, de Almeida, and Nóbrega 2018), growing evidence supports therapeutic development toward a number of generalizable polyQ pathogenic pathways, including disrupted UPS, autophagic dysfunction, overextended cellular stress pathways, and impaired DNA damage repair (Wang 2018). It should be noted, that all of these pathways are not exclusive to neurons, but rather contribute to universal functions within all CNS cells. Therefore, development of any therapeutic intervention will need to consider target engagement across multiple cell types.

The most obvious target for therapeutic intervention is suppression or modification of the mutant protein in order to ameliorate disease phenotypes. The lack of overt phenotypes following germline or conditional knockout of endogenous ATXN3 in mice suggests that ATXN3 is not an essential protein in mammals (Boy et al. 2009; Schmitt et al. 2007). Thus, reducing total levels of ATXN3 protein would likely be well tolerated in SCA3 patients. To target a causative gene, two main types of oligonucleotide-based therapeutic strategies are currently used: RNA interference (RNAi) and antisense oligonucleotides (ASO). Both strategies have been used in SCA3 animals models, leading to differing levels of success depending on the delivery, target, and timing of therapeutic intervention (Moore et al. 2017; McLoughlin et al. 2018; Costa et al. 2013; Rodriguez-Lebron et al. 2013; Alves et al. 2008; Alves et al. 2010; Toonen et al. 2016; Toonen et al. 2017; Nobrega et al. 2013). ASOs in general have moved more quickly into human clinical trials for neurodegenerative diseases (Bennett, Krainer, and Cleveland 2019). The first ASO-mediated gene modifying therapy gained FDA-approval for the treatment of spinal muscular atrophy (SMA) in 2016 (Finkel et al. 2016; Finkel et al. 2017). The positive safety profile and extraordinary efficacy of ASOs in SMA has boosted interest in ASO treatment of other genetic neurodegenerative diseases. ASO targeting the *HTT* transcript for the treatment of HD is currently in phase 3 clinical trials, following several successful preclinical studies and a phase 1/2a clinical trial (Lane et al. 2018; Tabrizi et al. 2019; Kordasiewicz et al. 2012). The success and continued clinical development of ASOs as potential treatment for other genetic neurodegenerative diseases solidify ASOs as a feasible and potentially powerful therapy for SCA3 and other polyQ diseases.

Many therapeutic interventions downstream of the mutant protein have been tested in SCA3 disease. SCA3 therapies that target protein aggregation and stimulate protein clearance through UPS and autophagy were recently summarized in a review (Matos, de Almeida, and Nóbrega 2018). Toward the goal of impeding protein aggregation, heat shock proteins (HSP) have been extensively studied as polyQ protein chaperones that could play a protective role by refolding mutant proteins and increasing degradation; chaperones that have been studied include Hsp40, Hsp70, and Hsp90 (Warrick et al. 2005; Bilen and Bonini 2007; Silva-Fernandes et al. 2014). Pharmacological inhibitors of Hsp90 (e.g.17-AAG and 17-DMAG) that alleviate inhibition of heat shock transcription factor 1 (HSF1), have been shown to reduce aggregation and toxicity in SBMA mouse models (Rusmini et al. 2011), and in SCA3 C. elegans and mouse models, (Silva-Fernandes et al. 2014). These studies reveal the therapeutic potential for Hsp90 inhibitors via a HSF1-dependent mechanism in SCA3 and perhaps other polyQ diseases. More recently DNAJC8 (Ito et al. 2016), HSPB7 (Wu et al. 2019), and the novel *Dictyostelium* chaperone, SRCP1 (Santarriaga et al. 2015; Santarriaga et al. 2018) have been described as potential chaperone regulators of the early polyQ aggregation steps. While there is strong evidence for pathogenic dysregulation of HSF1 and downstream chaperones in many neurodegenerative diseases, including HD, Alzheimer's Disease and Parkinson's disease (Gomez-Pastor, Burchfiel, and Thiele 2018), further testing in vertebrate SCA3 animal models of HSF1 activators and novel chaperones that directly impede polyQ aggregation will be required to determine the therapeutic utility of chaperone treatments.

Protein clearance therapies have also been explored extensively in SCA3 and the broader polyQ disease field with some success. In the context of autophagy, genetic Beclin1 overexpression or pharmacological manipulation by Rapamycin to enhance autophagy has been shown to be neuroprotective in cell and animal models. Some caution is required toward dosing and target, however, as a recent combinatorial therapy with mTOR-inhibitor and Lithium Chloride, autophagy inducers, led to increased neurotoxicity in SCA3 mice (Duarte-Silva et al. 2016).

Studies of SCA3 patient serum and cellular models have defined increased levels of reactive oxygen species and decreased antioxidant capacity (de Assis et al. 2017). Increasing antioxidant levels to reduce oxidative stress and subsequent DNA damage levels could be therapeutic in polyQ diseases, as has already been shown to be the case in ATM-deficient mice (Browne et al. 2004). Oxidative stress is known to increase nuclear

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localiza nto the nucleus might be therapeutically beneficial.

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