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REVIEWS

Review

Poly(ADP-Ribosylation) in Age-Related Neurological Disease

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A central and causative feature of age-related neurodegenerative disease is the deposition of misfolded proteins in the brain. To devise novel approaches to treatment, regulatory pathways that modulate these aggregation-prone proteins must be defined. One such pathway is post-translational modification by the addition of poly(ADP-ribose) (PAR), which promotes protein recruitment and localization in several cellular contexts. Mounting evidence implicates PAR in seeding the abnormal localization and accumulation of proteins that are causative of neurodegenerative disease. Inhibitors of PAR polymerase (PARP) activity have been developed as cancer therapeutics, raising the possibility that they could be used to treat neurodegenerative disease. We focus on pathways regulated by PAR in neurodegenerative disease, with emphasis on amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration (FTD).

Highlights

ADP-ribosylation activity is essential for brain development, function, and integrity.

PAR promotes the disease-like aggregation of proteins causative of human neurodegenerative diseases such as ALS and FTD.

Inhibition of PARP activity mitigates the neurotoxicity of ALS- and FTD-causing proteins to mammalian primary neurons in culture and to *Drosophila*.

ADP-Ribosylation

Post-translational modifications are fundamental to regulating protein function and stability in all domains of life. One such modification is ADP-ribosylation (Figure 1A) – the rapid transfer of **ADP-ribose (ADPr)** (see Glossary) onto a target protein from βNAD$^+$ [1–3]. ADPr-transferase activity has independently evolved in three protein families: the sirtuins, the bacterial TM1506 family, and the **poly(ADP-ribose)** (PAR) polymerases, the latter being the principle ADPr-transferase system in humans [4,5]. The human **poly(ADP-ribose) polymerase** (PARP) superfamily consists of 17 proteins with a canonical ADPr-transferase (ART) domain, and each has specific enzymatic activities: 11 are mono(ADPr) transferases (PARP-3, -4, -6, -7, -8, -10, -11, -12, -14, -15, and -16) that link a single ADPr onto the target protein (**MARylation**); PARP-1, -2, -5a, and 5b have the capacity to add sequential ADPr subunits and generate chains of poly(ADP-ribose) (**PARylation**); the remaining two (PARP-9 and -13) are enzymatically inactive [6]. Amino acids known to be conjugated by ADPr are Arg, Asp, Cys, Glu, Lys, Ser, and Tyr [2,7]. ADP-ribosylation is a labile modification, in part due to the activity of the ADPr hydrolases.

In humans there are more than eight ADPr hydrolases, and each has specific protein domains and substrates (Figure 1B,C). For example, PAR is cleaved at the ribose–ribose bond by **poly (ADP-ribose) glycohydrolase** (PARG) and to a lesser extent by ADPr glycohydrolase 3 (ARH3). However, while ARH3 can remove the terminal ADPr conjugated to a serine residue, PARG cannot hydrolyze the terminal ADPr, and leaves behind a MARylated protein [8–11]. **MacroD1 and MacroD2** reverse MARylation at acidic residues; similarly, ADPr glycohydrolase OARD1 (also known as **terminal ADP-ribose protein glycohydrolase 1**, TARG1) hydrolyzes ADPr conjugated to acidic residues to remove either a single ADPr subunit or the entire PAR chain [12–14]. ADPr and PAR can also be converted into phosphoribose by the hydrolysis of the two phosphate groups in ADPr by nucleoside diphosphate-linked moiety X-type motif 16 (NUDT16) and ectonucleotide pyrophosphatase/phosphodiesterase family member 1 (ENPP1) [15–17]. Because of the dynamic and reversible nature of ADP-ribosylation, it is often central to stress pathways where there is a need for a rapid response combined with tightly controlled termination of the signaling pathway.

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In mammals, the most widely studied stress-induced function of ADP-ribosylation is in the **DNA damage response** (DDR). At sites of DNA damage, PARP-1, a nuclear enzyme, modifies itself (automodification) and target proteins (transmodification). DNA repair enzymes, such as the DNA repair protein XRCC1, non-covalently bind to the PAR polymer and promote the localization of repair proteins to the sites of damaged DNA [18,19]. Finally, to complete the repair of damaged DNA, PARG is recruited and the PAR polymer is digested [20]. Upon excessive activation of PARP-1, PAR is cleaved and translocated into the cytoplasm where it triggers a cell-death mechanism called **parthanatos** (see below). Thus, ADP-ribosylation regulates the modified protein, as well as the proteins that bind to PAR, and isolated PAR functions as a signaling molecule. PARylation by the PARP superfamily is now known to regulate many fundamental cellular processes including chromatin compaction and gene expression, protein stability and cellular signaling, and telomeres and the mitotic spindle (reviewed in [21–26]).

ADP-ribosylation was initially pursued as a cancer therapeutic; however, it is becoming increasingly clear that ADP-ribosylation also regulates many features associated with age-related brain disorders, including DNA damage, protein localization and aggregation, and cell death. Hence, it has been proposed that PARP inhibitors developed as cancer treatments could be repurposed for other disorders, including neurological diseases [27]. We discuss here recent work that implicates ADP-ribosylation activity as being crucial for age-related brain function, and in the following we focus on how ADP-ribosylation relates to ALS and FTD, and discuss future considerations.

The Importance of Regulating ADP-Ribosylation in the Brain

Mounting evidence implicates ADP-ribosylation as a key pathway required for normal brain function. **Copy-number variants** (CNVs) and intronic **single-nucleotide polymorphisms** (SNPs) at the ADPr-hydrolase *MACROD2* locus have been identified in epilepsy, autism spectrum disorder (ASD), ischemic stroke, multiple sclerosis, and schizophrenia [28–39]. Non-coding SNPs can regulate local genes, long-range genes, and genes positioned on different chromosomes, and intronic SNPs in *MACROD2* have been shown to affect to transcriptional clusters on several other chromosomes [40]. It remains to be elucidated whether the intronic SNPs and CNVs contribute to disease via misregulation of *MACROD2* or of genes elsewhere in the genome. However, in six independent families, recessive and inactivating mutations in the ADPr hydrolase ARH3 (*ADPRHL2*) gene were identified in patients that suffer from early-onset neurodegeneration [41]. Affected individuals experience stress-induced epilepsy, ataxia, and death within the first decade of life [41]. Recessive and inactivating mutations have also been identified in the gene (*OARD1*) encoding TARG1 [14]. Affected individuals from an extended family experienced severe neurodevelopmental delay, seizures, and motor deficits [14]. TARG1 removes ADPr from Glu and Asp residues, whereas ARH3 hydrolyzes PAR and ADPr from Ser residues [9–14] (Figure 1). The clinical presentation of patients harboring inactivating mutations in TARG1 and ARH3 suggests that failure to reverse ADP-ribosylation at Ser, Glu, and Asp residues is catastrophic for human brain function and results in rapid and severe neurological dysfunction.

ADP-ribosylation is also implicated in neurodegenerative disease characterized by abnormal protein aggregation. For example, proteins linked to the motor neuron disease ALS, FTD, and Parkinson's disease (PD) (TDP-43, FUS, and α-synuclein, respectively) are PAR-binding proteins, and PAR regulates their cellular localization, aggregation, and associated neurotoxicity [42–46]. Inhibiting the activity of PARP-1 and PARP-2 (collectively referred to as PARP-1/2), as well as of PARP-5a and PARP-5b (PARP-5a/5b), is beneficial in a variety of cellular, neuronal, and rodent models of ALS, FTD, and PD [43,45–47]. In addition, in cellular and animal models of ischemic stroke, downregulation or small-molecule inhibition of PARP-1 profoundly mitigates neural loss [48–50]. Collectively, these studies suggest that ADP-ribosylation facilitates disease progression. Indeed, inactivating mutations in *XRCC1* lead to ataxia in the fifth decade of life [51]. XRCC1 protein is localized to damaged

Glossary

ADP-ribose (ADPr): ADP-ribose is generated by the hydrolysis of βNAD$^+$ and is covalently linked to target proteins.
Copy-number variants (CNVs): duplications or deletions of sections of the genome. There is variation in CNVs in the general population and some are positively associated with disease.
DNA damage response (DDR): this is initiated upon detection of a DNA lesion, such as a single-strand break (SSB), a double-strand break (DSB), mismatches, insertion/deletion loops, and abnormal DNA bases.
MacroD1 and MacroD2: mono(ADP-ribose) hydrolases that cleave and remove the terminal ADP-ribose from the target protein.
MARylation: the addition of a single ADPr subunit to a target protein via the hydrolysis of βNAD$^+$.
PAR-binding motif (PBM): a consensus motif consisting of hydrophobic and basic residues that bind to the negatively charged PAR polymer.
Parthanatos: a caspase-independent cell death mechanism that is triggered by the release of PAR into the cytoplasm. The name is derived from Greek mythology: Thanatos is the personification of death.
PARylation: the covalent addition of sequential ADPr moieties onto an acceptor protein to generate linear and sometimes branched polymers of poly (ADPr).
Poly(ADP-ribose) (PAR): a post-translational modification comprising multiple ADPr molecules in a single chain or in branched forms.
Poly(ADP-ribose) polymerases (PARPs): an enzyme family characterized by the presence of an ADPr-transferase catalytic domain. Within the family, the 17 PARPs have structural, enzymatic, and functional differences.
Poly(ADP-ribose) glycohydrolase (PARG): an enzyme that hydrolyzes PAR at glycosidic linkages to form free ADPr molecules.
Single-nucleotide polymorphism (SNP): a nucleotide in the DNA sequence that differs from that of the general population. Some SNPs are significantly correlated with disease.
Single-strand breaks (SSBs): lesions in DNA that affect one strand of DNA and can elicit the DNA-damage response.

DNA by binding to the PAR scaffold generated by PARP-1 [18,19]. Loss of *XRCC1* leads to sustained PARylation, impaired repair of **single-stranded DNA breaks** (SSBs), and neuronal loss in mice that is rescued by deletion of *Parp1* [51], suggesting that XRCC1 mutations lead to ataxia via activation of PARP-1. Thus, ADP-ribosylation regulation of brain disease could be considered a spectrum in which mutations in the ADPr-hydrolase enzymes cause extreme and early-onset neurological disease, whereas low-level but perhaps long-term PARP activation by disease-causing factors promotes age-related brain dysfunction.

Terminal ADP-ribose protein glycohydrolase 1 (TARG1): an enzyme that cleaves the ester bond between the terminal ADPr molecule and the target protein.

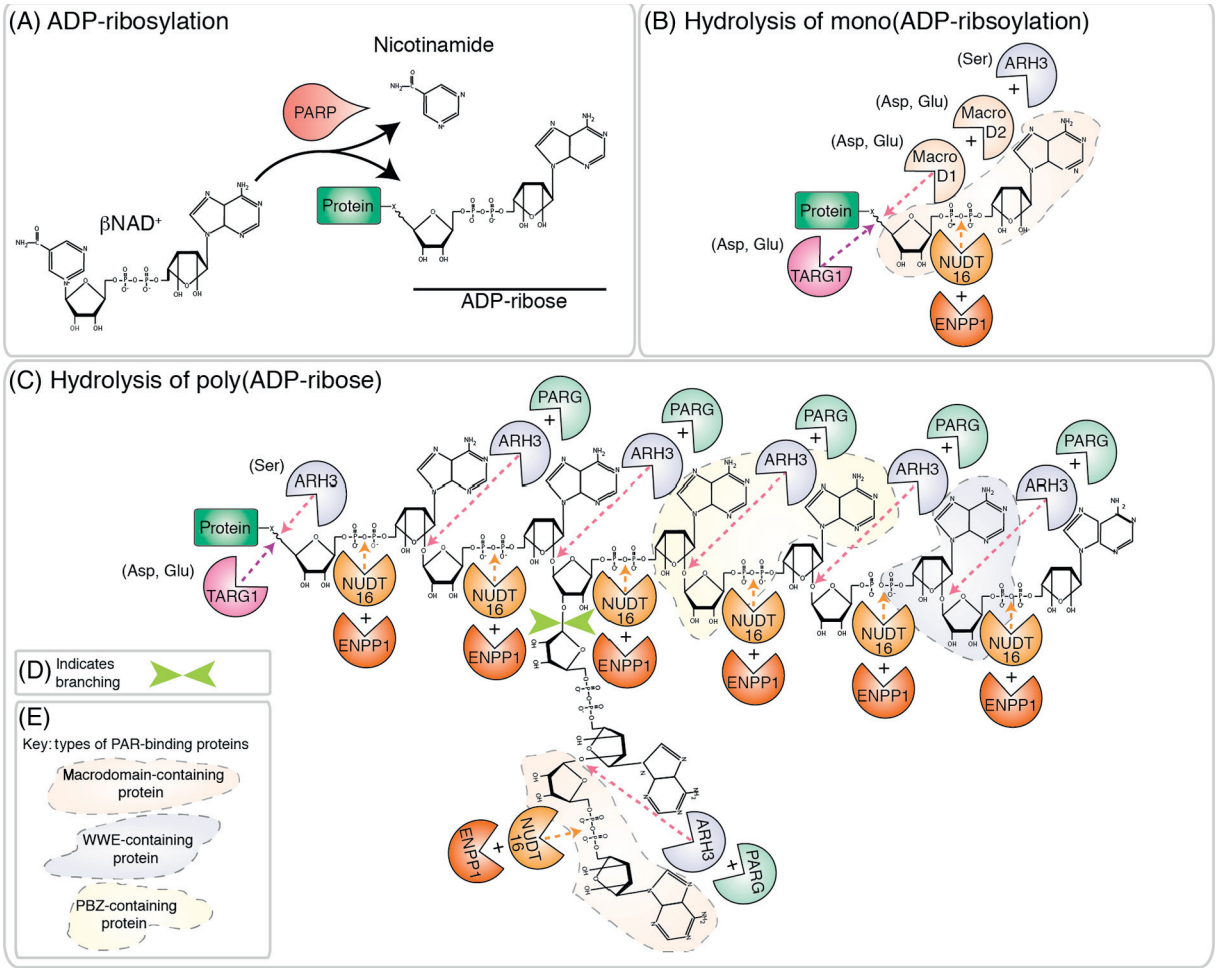
Seminal findings on the role of PARP-1 in parthanatos have emerged from studies in disease models of ischemic stroke and PD (see below). The role of ADP-ribosylation in promoting protein aggregation in diseases such as ALS, FTD, and PD is a more recent concept. Many of the disease pathways linked in ALS/FTD (reviewed in [52,53]) are regulated by ADP-ribosylation, including (i) DNA damage, (ii) protein localization, (iii) protein aggregation, and (iv) cell death. In the following sections we discuss the pathways regulated by ADP-ribosylation in relation to ALS/FTD and PD. We will consider the benefits and challenges in deciphering the potential of targeting ADP-ribosylation activity as a therapeutic strategy.

ALS/FTD and DNA Damage

Brain disorders share remarkable overlap in genetics and affected pathways. Two exemplary diseases are ALS and FTD, which are collectively known as ALS/FTD (Figure 2 and Box 1). In >95% of ALS and ~45% of FTD the normally nuclear protein TDP-43 is mislocalized to the cytoplasm where it accumulates as a phosphorylated protein in ubiquitin-positive inclusions (Figure 3 and Box 1). A second notable gene is *FUS* (fused in sarcoma), whose protein product is mislocalized to the cytoplasm of affected brain regions in <4% ALS and ~5% of FTD (Box 1). FUS is an RNA-binding protein that localizes to sites of DNA damage in a manner that is dependent upon PARP-1 (reviewed in [54]). More recently, TDP-43 was shown to facilitate the repair of damaged DNA [55,56]. Thus, the loss of TDP-43 and FUS from the nucleus in ALS/FTD could impact the repair of damaged DNA in disease. Analysis of post-mortem tissue indicates that some of the early signs of DNA damage are present in the ALS spinal cord [46,57,58], suggesting that, at the endpoint of ALS, motor neurons may be undergoing increased levels of DNA damage and/or their ability to repair damaged DNA is compromised. Increased DNA damage has also been detected in human induced pluripotent stem cell (iPSC)-derived motor neurons generated from a variety of ALS genetic backgrounds [59–62]. However, it is unclear if DNA damage plays a vital role in disease progression.

ALS-associated mutations that occur in the nuclear localization sequence (NLS) of FUS reduce the recruitment of the protein to sites of DNA damage to varying degrees [58,60,63–65], alter key protein interactions required for the repair of DNA [58,65,66], and lower the efficiency of DDR in proliferating cells and human iPSC-derived motor neurons [58,61,63,64]. However, although mutations in the NLS of FUS cause neurodegenerative phenotypes in the mouse [66,67], DNA damage is only detected in the ALS-FUS mouse model with the most aggressive neurodegenerative symptoms [66]. It is important to note that the RGG domain of FUS directly binds to PAR and also localizes the protein to sites of DNA damage [42,63]. It is possible that mutations in the PAR-binding region (the RGG domain) of FUS may have a greater capacity to misregulate the function of the protein in the repair of damaged DNA.

It remains to be determined whether targeting the DDR could be a potential therapeutic strategy in ALS/FTD. PARP-1/2 inhibitors mitigate the neurotoxicity of TDP-43 [46,47], suggesting that downregulation of a response to DNA damage may be beneficial; however, PARP-1/2 regulates many pathways. As discussed below, activation of PARP-1 promotes PAR-mediated protein



Trends in Genetics

Figure 1. ADP-Ribosylation. (A) Poly(ADP-ribose) polymerases (PARPs) catabolize βNAD$^+$ into nicotinamide and ADP-ribose (ADPr), and the resulting ADPr is covalently linked to an acceptor protein. The addition of a single ADPr subunit is called mono(ADP-ribosylation) (MARylation). Amino acids targeted are Arg, Asp, Cys, Glu, Lys, Ser, and Tyr [2,7]. (B) Removal of a single ADPr subunit from acidic residues (Asp and Glu) is mediated by MacroD1, MacroD2, and TARG1 [12–14]. ARH3 removes the ADPr from Ser residues [10]. NUDT16 and ENPP1 cleave the two phosphate groups in ADPr to generate a phosphoribosylated protein [15–17]. (C,D) Poly(ADP-ribosylation) (PARylation) involves sequential addition of ADPr to generate linear and sometimes branched (D, green arrows) polymers of poly(ADP-ribose) (PAR). PARylation is reversed by PARG-mediated hydrolysis of the ribose–ribose bond. ARH3 also cleaves the ribose–ribose bond but, unlike PARG, ARH3 can also remove the protein-proximal ADPr from Ser residues in the target protein [8–11]. TARG1 removes the entire PAR chain by cleavage of the protein-proximal ADPr [14]. The two phosphate groups in ADPr are hydrolyzed by NUDT16 and ENPP1 [15–17]. (E) Conserved protein domains that recognize and bind to specific sites of PAR polymers (reviewed in [115]). These domains include the PBZ domain, the WWE domain, the macrodomain, and the PAR-binding motif (PBM). The physical contacts that are made with the ADPr moiety are known for the PBZ domain, the WWE domain, and the macrodomain. Regions of the PAR chain are shaded in (B) and (C) according to the key in (E). It is currently unknown what the PBM interacts with in PAR.

aggregation and cell loss. In the following sections we discuss abnormal protein localization and cell death in ALS/FTD and the role that PAR may play in these processes.

Protein Mislocalization in ALS/FTD

The disease-associated pathology of TDP-43 and FUS suggest that nucleocytoplasmic transport is affected in disease, and agents that can relocalize TDP-43 and FUS back to the nucleus are therefore of therapeutic interest (reviewed in [68]). Studies that launched from *Drosophila* uncovered that a reduction of cytoplasmic (PARP-5a/5b) ADP-ribosylation activity decreases the accumulation of TDP-43 in the cytoplasm and mitigates TDP-43 neurotoxicity [43]. In addition,

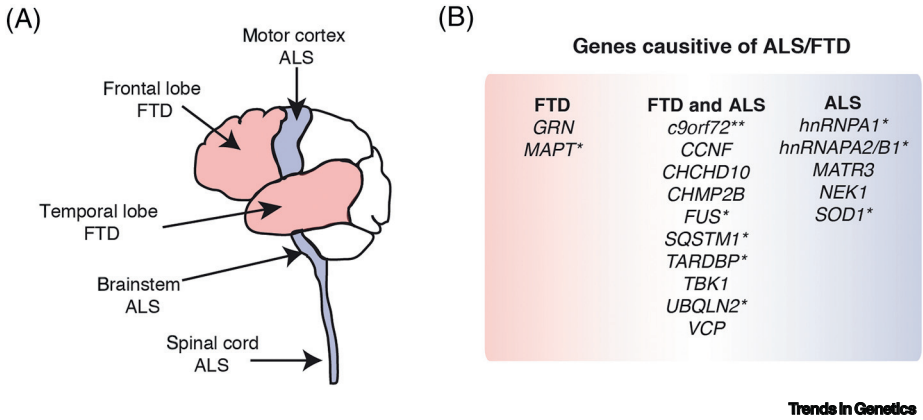


Figure 2. The Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Degeneration (FTD) Disease Spectrum. (A) At autopsy many brain regions can present with neuronal loss and pathology in ALS and FTD. However, the most consistently affected regions in ALS are the motor cortex, brainstem, and spinal cord, and for FTD the frontal and temporal cerebral lobes (reviewed in [98,111]). (B) Genes that when mutated give rise to either ALS, FTD, or to both ALS and FTD (reviewed in [52]). *Indicates genes whose protein products are known to aggregate in disease tissue. **The mutation in *c9orf72* is an intronic G_4C_2-hexanucleotide expansion that gives rise to abnormal accumulation of G_4C_2-hexanucleotide-containing RNA and dipeptide-repeat proteins that are translated from the intronic repeat. (reviewed in [52,53]

inhibition of nuclear ADP-ribosylation (PARP-1/2) mitigates TDP-43-associated neurotoxicity in rodent neurons and reduces arsenite-induced accumulation of TDP-43 in the cytoplasm of mammalian cells [44,47]. Furthermore, upon treatment with hydrogen peroxide, inhibition of PARP-1/2 inhibits cytoplasmic accumulation of FUS [69]. Collectively, these data indicate that PARP-1/2 and PARP-5a/5b can regulate the nuclear and cytoplasmic levels of TDP-43 and FUS. PARP-1 regulates nucleocytoplasmic localization of several proteins (reviewed in [70]). It remains to be seen how PARP-1/2 promotes TDP-43 and FUS accumulation in the cytoplasm, whether it involves a nuclear export mechanism, and whether PARP-1/2 works together with PARP-5a/5b.

There is, however, evidence to suggest that stress is involved in TDP-43 pathology in the ALS spinal cord [71–73]. ADP-ribosylation activity is widely recognized as a stress-induced protein modification (see above). In the following section we discuss the role of stress and the ways in which stress-activated ADP-ribosylation may regulate cytoplasmic protein aggregation in ALS/FTD.

Box 1. The Pathophysiology of ALS and FTD

ALS and FTD (see Figure 2 in main text) are fatal disorders for which there are few treatments and no cures. ALS is a motor neuron disease that commonly occurs in midlife; patients lose all motor control and typically die within 2–5 years of symptomatic onset (reviewed in [98]). In >95% of ALS the normally nuclear protein TDP-43 is cleared from the nucleus (see Figure 3 in main text) and instead accumulates as a phosphorylated protein in ubiquitin-positive inclusions in the cytoplasm (reviewed in [98]). For the most part ALS is apparently sporadic; however, ~10% of cases are the result of inherited genetic mutations (reviewed in [98]). *SOD1* (superoxide dismutase) was the first gene found to be mutated in ALS; however, along with patients that harbor mutations in *FUS* (fused in sarcoma), these patients lack TDP-43 pathology [106–108]. TDP-43 pathology is the hallmark of all other disease backgrounds, suggesting that disease-causing factors converge on TDP-43.

TDP-43 pathology is also observed in ~45% of FTD [106,109,110]. FTD, the second most common presenile dementia, is characterized by degeneration of the frontal and temporal lobes (see Figure 2A in main text). FTD leads to changes in behavior, language, and personality, leading to death within ~10 years of diagnosis. Up to 50% of FTD cases are the result of inherited mutations (reviewed in [111]). Intriguingly, at least 10 genes are shared by ALS and FTD (see Figure 2B in main text), including the most frequently affected gene, *c9orf72* [52,112,113]. The clinical, genetic, and pathological overlap between ALS and FTD has led to the understanding that these two diseases are on opposing ends of a shared ALS/FTD disease spectrum [52]. TDP-43 pathology can also be observed in Alzheimer's, Pick's, Alexander's disease, and hippocampal sclerosis (reviewed in [114]), suggesting that dysfunction in multiple situations can impact on pathways that converge on TDP-43.

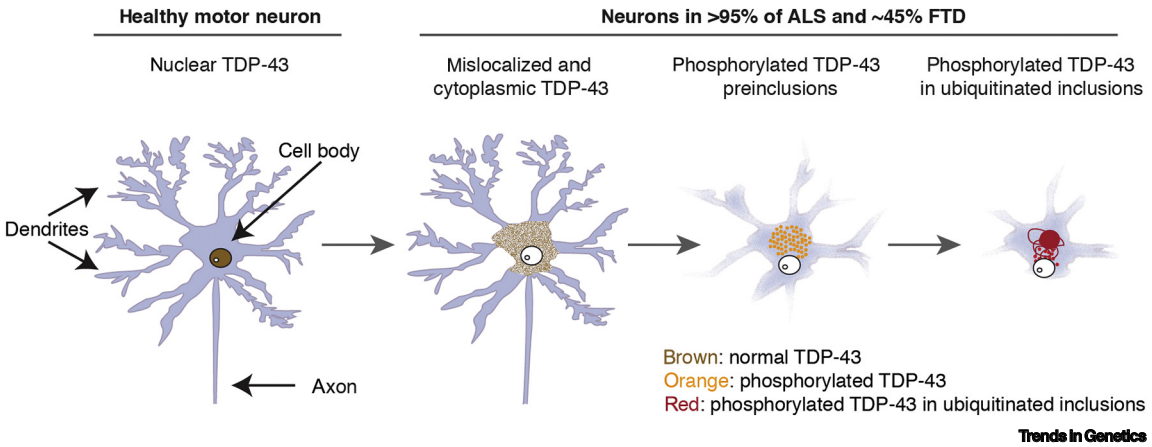


Figure 3. TDP-43 Pathology in Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Degeneration (FTD). In healthy neurons TDP-43 (brown) is nuclear. In >95% of ALS, and in ~45% of FTD, TDP-43 is cleared from the nucleus and accumulates in the cytoplasm. Cytoplasmic TDP-43 concentrates at phosphorylated foci (orange) that mature into ubiquitinated inclusions (red) [106,109,110].

Protein Aggregation in ALS/FTD

Some proteins have an intrinsic capacity to oligomerize into high-molecular-weight protein fibrils, and these proteins, including TDP-43 and FUS, often harbor an intrinsically disordered region (IDR) (reviewed in [74]). Protein fibrilization can be essential for protein function but it can also lead to the precipitation of the protein into insoluble and dysfunctional protein aggregates (reviewed in [75]). Elucidation of the biophysical properties that regualte both normal and abnormal protein fibrilization is providing crucial insight into the progression of neurodegenerative disease. A thermodynamic phenomenon linked to normal and abnormal protein fibrillization is liquid–liquid phase separation (LLPS). LLPS occurs when inter- and intramolecular protein interactions are stronger than the interactions between the protein and the surrounding liquid, such that protein solubility is reduced and LLPS is promoted (reviewed in [76]). Multivalent interactions determine the phase-separation properties of a protein, and influencing factors include protein domains, for example an IDR (reviewed in [77]), as well as extrinsic factors including osmolarity and interactions with charged molecules such as nucleic acids and PAR (Figure 4A). The role of PAR in localizing proteins is well established; however, more recently it has been proposed (see below) that PAR may localize proteins by promoting LLPS.

In the cell, LLPS governs the formation of non-membrane-bound, RNA-rich organelles, including P bodies and stress granules (reviewed in [78]), the latter being enriched for PAR [43,79,80]. Stress granules inhibit global translation by sequestering translation preinitiation complexes in the cytoplasm during stress (reviewed in [81]). Several mechanisms regulate stress granules, including interactions mediated by IDR domains (reviewed in [78]). Bioinformatic analyses have revealed overlap between PAR-binding and ADP-ribosylated proteins with IDR-containing proteins and proteins that localize to stress granules [42,82,83], suggesting that PAR may influence LLPS of IDR-containing proteins. Indeed, stress-granule proteins have been shown to be PARylated in cells exposed to chemical stressors [47,79]. It is not known which of the PARPs promote PARylation of stress-granule proteins or where the PARylation takes place in the cell. It is known that five PARPs (PARP-5a, -12, -13.1, -13.2, and -14) localize to stress granules [43,79,80], that PARP-1 activity promotes stress-granule formation [46,47,80,84], and that PARP-12 translocates from the Golgi to stress granules in a manner dependent upon PARP-1 activity and its WWE domain (a PAR-binding module) (Figure 1) [80]. Moreover, after the removal of stress, stress granules resolve; however, upon inhibition of PARG, stress granules persist [47,79],

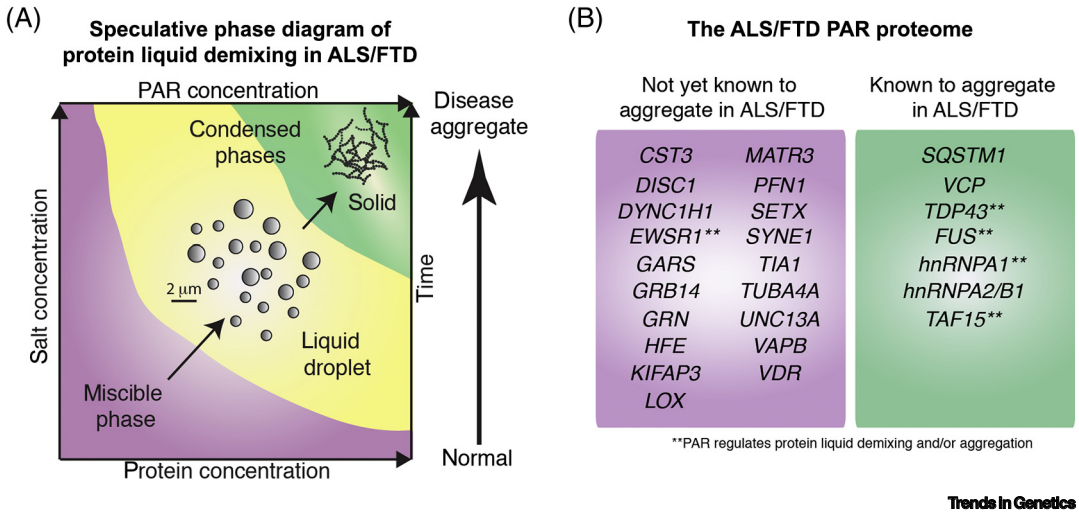


Figure 4. Amyotrophic Lateral Sclerosis (ALS)/Frontotemporal Degeneration (FTD)-Associated Genes and Their Relationship to PARP Activity. (A) A speculative phase diagram representing the conditions that may regulate protein phase separation. Poly(ADP-ribose) (PAR) promotes liquid–liquid phase separation of TDP-43, FUS, and hnRNAPA1 [43,47,85]; however, long-term incubation of PAR with FUS, TAF15, and EWSR1 promotes protein aggregation [42]. Thus, PAR-mediated phase separation may initially promote phase separation, but with time disease-like protein fibrils are formed. (B) Proteins of ALS/FTD that are known to be PARylated or interact with PAR. The 128 genes and corresponding proteins from the ALS online genetics database (http://alsod.iop.kcl.ac.uk/) of genes associated with ALS, and that are causative for ALS and FTD, plus the FTD genes *TIA1* and *KIF5A*, were cross-referenced to the PAR proteome [84,116–119]. Proteins identified as being directly PARylated or non-covalent binders of PAR are listed, and are grouped into proteins that are not yet known to aggregate in disease and those that are known to aggregate in disease.

suggesting that PARG promotes stress-granule dissolution. In the *in vitro* and cellular setting, PAR promotes LLPS of TDP-43, FUS, and hnRNPA1 [42,43,47,85], and *in vitro*, PARG can dissolve FUS aggregates localized to DNA damage [69], These studies suggest that in the cell PAR promotes localized phase separation in stress granules, as well as at sites of DNA damage.

Many of the proteins that cause ALS/FTD, including TDP-43, localize to stress granules (reviewed in [86]), it was therefore proposed that stress granules may be the initial seed for abnormal TDP-43 aggregation. TDP-43 harbors a **PAR-binding motif** (PBM), which when mutated prevents PAR binding and TDP-43 recruitment to stress granules [43]. TDP-43 that is unable to bind to PAR forms aberrantly phosphorylated protein aggregates that are excluded from stress granules [43], suggesting that PAR-dependent recruitment of TDP-43 to stress granules protects the protein from disease-associated phosphorylation. The finding that TDP-43 aggregates that form outside stress granules are pathological has been confirmed in independent studies that have also demonstrated that phosphorylated TDP-43 aggregates are neurotoxic [87–89]. Collectively, these studies indicate that PAR-mediated aggregation of TDP-43 is important for preventing disease-associated aggregation, and suggest that, in conditions where βNAD^+ and PAR may be limiting, stress may give rise to neurotoxic aggregation of TDP-43.

An increased concentration of ALS/FTD-associated proteins in liquid droplets can promote pathological fibrillization [85,90–92]. Thus, LLPS provides an environment for aggregation-prone proteins to fibrillize and mature into insoluble precipitates over time. In the cell, exposure to chronic stress leads to the dissolution of stress granules and the formation of phosphorylated TDP-43 aggregates akin to those observed in disease [43]. Thus, although PAR recruitment to stress granules is initially protective (see above), PAR is an early seeding agent of protein-rich liquid droplets that with time can transition into insoluble disease-like aggregates (Figure 4A). Indeed, long-term incubation of FUS, TAF15, and EWSR1 with PAR leads to the formation of disease-like

aggregates *in vitro* [42]. ADP-ribosylation in ALS/FTD could be considered a catch-22: it facilitates the localization of protein into soluble and protective environments – that unfortunately also have the capacity to transition the proteins into toxic species. A therapeutic strategy could be to ensure proper stress-granule localization and the timely resolution of stress granules [43]. Long-term activation of stress and ADP-ribosylation activity is also known to lead to cell death. In the following section we discuss PAR-mediated cell death and ALS/FTD.

Hyperactivation of PARP-1 and Parthanatos

PARP-1 aids the response to stress by regulating cell survival or cell death. The defining factor correlates with the extent to which PARP-1 is automodified. Upon hyper-automodification

Key Figure

Model for ADP-Ribosylation in Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Degeneration (FTD)

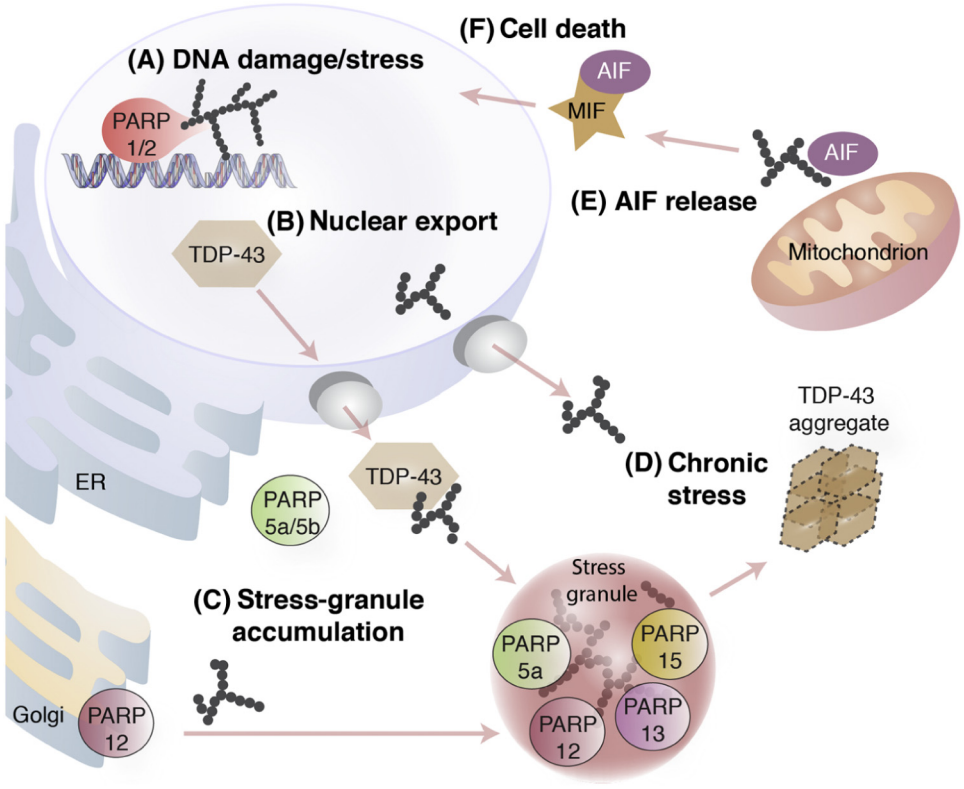


Figure 5. (A) Upon activation of stress pathways, PARP-1 becomes automodified with poly(ADP-ribose) (PAR). (B) Stress-induced accumulation of TDP-43 in the cytoplasm is promoted by PARP-1/2 and PARP-5a/5b activity. (C) The activity of PARP-1 promotes cytoplasmic stress-granule formation. PARP-12 translocates from the Golgi to stress granules where it accumulates alongside PARP-5a, -12, -13, and -15. TDP-43 is recruited to stress granules by binding to PAR. (D) Under conditions of chronic stress, TDP-43 aggregates are formed that lack stress-granule proteins and are phosphorylated. (E) Chronic stress also induces PAR-mediated release of apoptosis-inducing factor (AIF) from mitochondria. It is unknown whether the chronic stress that leads to TDP-43 aggregation also leads to AIF release from mitochondria. (F) AIF in the cytoplasm binds to and forms a protein complex with macrophage migration inhibitory factor (MIF), which translocates to the nucleus where MIF digests the genomic DNA, leading to death of the cell. Abbreviation: ER, endoplasmic reticulum.

of PARP-1, PAR is cleaved and released into the cytoplasm where it elicits a caspase-independent cell death mechanism called parthanatos (reviewed in [93]). Parthanatos has been studied for more than two decades, and much of our understanding has come from cellular and animal models of PD and ischemic stroke, the latter of which is associated with glutamate excitotoxicity (reviewed in [94]). Glutamate excitotoxicity leads to hyperactivation of PARP-1 and subsequent translocation of PAR from the nucleus to the cytoplasm, where it binds to apoptosis-inducing factor (AIF), releasing the protein from the mitochondria and into the cytoplasm [95]. In the cytoplasm, AIF binds to the nuclease macrophage migration inhibitory factor (MIF), both proteins translocate into the nucleus, and MIF digests the genomic DNA, triggering cell death [96]. The protein(s) that mediate transport of PAR into the cytoplasm and to the mitochondria during parthanatos remain enigmatic. Reducing PARP-1 in cellular and rodent models of stroke mitigates neuronal toxicity [45,48–50], indicating that PARP-1 activation is important for disease progression.

In a mouse model of PD, preformed fibrils of α-synuclein (α-syn) injected into the cortex led to excitotoxicity, PAR-induced aggregation of α-syn, and neurotoxicity that is mitigated by inhibition of PARP-1/2 [45]. These studies suggest a feedforward mechanism whereby the excitotoxicity induced by preformed α-syn fibrils hyperactivates PARP-1, which leads to the release of PAR into the cytoplasm where it interacts with α-syn, promoting more aggregation and toxicity, which in turn further activates PARP-1. Intriguingly, measurements of the cerebrospinal fluid (CSF) of a cohort of PD patients revealed elevated levels of PAR compared to controls [45], suggesting that PAR could be a potential biomarker for PD. Elevated levels of PAR have also been detected in the nuclei of spinal cord motor neurons in ALS patients [46]; is it possible that a similar mechanism regulates TDP-43 and that PAR is a potential CSF biomarker of ALS/FTD.

Neuronal excitotoxic mechanisms known to activate PARP-1 in parthanatos, such as glutamate excitotoxicity, ER stress, mitochondrial stress, and increased ROS [93], have been widely implicated in ALS/FTD (reviewed in [97,98]). For example, glutamate excitotoxicity was one of the first pathways implicated in ALS (reviewed in [98]). FTD- and ALS-causative mutations occur in genes involved in ER homeostasis (valosin-containing protein, *VCP*) (reviewed in [52]) and in mitochondrial ROS production (superoxide dismutase, *SOD1*) (reviewed in [98]). Could PARP activation link the stress pathways activated in disease to TDP-43 nuclear export, TDP-43 cytoplasmic aggregation, and neuronal death (Figure 5, Key Figure) – and if so how? For example, PARP-1/2 and PARP-5a/5b promote TDP-43 accumulation in the cytoplasm: is PAR the direct facilitator or does it act via an intermediary protein or proteins? Once in the cytoplasm, PAR incorporates TDP-43 into stress granules, but stress granules resolve under prolonged stress, leaving behind phosphorylated TDP-43: does the resolution of chronic stress granules also release PAR into the cytoplasm to initiate parthanatos? Because inhibition of PARP-1/2 and PARP-5a/5b mitigates toxicity in neuronal models, will the identification of pathways that activate ADP-ribosylation in disease provide insight into earlier intervention strategies? Finally, PAR is upregulated in motor neuron post-mortem spinal cord tissue, but is it also elevated in patient CSF? Addressing these questions may help to elucidate the potential of targeting ADP-ribosylation activity in ALS/FTD.

Concluding Remarks and Future Perspectives

Inhibition of ADP-ribosylation activity remarkably mitigates the neurodegenerative features in several animal disease models (Table 1), reinforcing the suggestion that PARP inhibitors, developed as cancer therapeutics, may be an option for the treatment of neurological disease [27]. We know very little of the role of ADP-ribosylation in the brain, such as which proteins are modified and how modification of these proteins regulates brain development, function, and integrity (see Outstanding Questions). Would long-term inhibition of PARP activity have a detrimental effect on the

Outstanding Questions

Which proteins are substrates of ADP-ribosylation in the brain, how does the modification modulate brain function, and how might their misregulation by ADP-ribosylation contribute to neurodegenerative disease?

Does increased ADP-ribosylation drive disease progression in ALS? Does this directly promote the nuclear export and aggregation of TDP-43?

Is disease-like aggregation of TDP-43 linked to parthanatos, and is parthanatos taking place in ALS/FTD?

Will PARP inhibitors be beneficial in ALS/FTD rodent models? How will the potential negative effects of PARP inhibitors be prioritized and balanced against the potential side effects of long-term PARP inhibition?

Table 1. Rodent Models of Neurodegenerative Disease That Have Shown Benefit from Therapeutic Inhibition of PARP-1/2[a]

Disease	Model	Inhibitors	Treatment	Sacrifice	Effect of PARP-1/2 inhibitor	Refs
AD	Rat: injection of Aβ(1–42) into hippocampal C3 region	Niacin	Daily i.p.i. for 7 days	7 days post-Aβ(1–42) injection	Reduced lipid peroxidation and ROS	[120]
HD	Mouse: transgenic for exon 1 of *HTT* with 150 CAG repeats driven by the human *HTT* promoter	INO 1001	Daily i.p.i. from 28 days onward	Not applicable	Improved lifespan and reduced motor deficits	[121]
Ischemic stroke	Mouse: MCAO and ACAO	INH_2BP	I.p.i. 2 h before occlusion	25 h/75 h after MCAO and ACAO	Inhibition of PARP-1/2 activity and reduced infarct size	[122]
Ischemic stroke	Mouse: MCAO	PJ34	I.p.i. 1 h and 2 h before occlusion	24 h and 72 h post-MCAO	Reduced iNOS expression and infarct size	[123]
Ischemic stroke	Mouse: MCAO	DPQ	I.p.i. 1 h and 2 h before occlusion	24 h and 72 h post-MCAO	Reduced infarct size	[123]
Ischemic stroke	Mouse: MCAO	DPQ	I.p.i. 2 h before and 2 h after occlusion	24 h post-MCAO	Reduced infarct size	[124]
PD	Mouse: i.p.i. MPTP 4 times at 1 h intervals	Benzamide	I.p.i. 30 minutes before and 90 minutes after the first MPTP injection	5 days after MPTP injection	Mitigated loss of tyrosine hydroxylase	[125]
PD	Mouse: unilateral injection of α-syn PFF into striatum	ABT-888	Mice were fed ABT-888	3 and 6 months after injection of α-syn PFF	Reduced motor deficits (3 months after α-syn PFF injection); mitigated loss of DA neurons (6 months after α-syn PFF injection)	[45]

[a]Abbreviations: ACAO anterior cerebral artery occlusion; AD, Alzheimer's disease; DA, dopaminergic neurons; DPQ, 4-dihydro-5-[4(1-piperidinyl)butoxyl]-1-(2H)-isoquinolinone; HD, Huntington's disease; INH_2BP, 5-iodo-6-amino-1,2-benzopyrone; iNOS, inducible nitric oxide synthase; i.p.i., intraperitoneal injection; MCAO, middle cerebral artery occlusion; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; PFF, preformed fibrils.

normal function of the brain, and how would long-term PARP inhibition impact on severe neurological disease? Perhaps the identification of ADP-ribosylated proteins in disease could lead to the generation of pathway-specific inhibitors with minimal side effects on other cellular pathways regulated by ADP-ribosylation.

In the past, uncovering the pathways regulated by ADP-ribosylation has been limited by the detection of the ADP-ribosylated substrates (proteins that are either directly modified or that bind to the ADPr or PAR modification), including conditions that maintain the modification and conditions that allow the identification of the substrates by mass spectrometry. Recent advances have been made in the development of methods that identify ADP-ribosylated targets [99–102] and that measure the levels of ADPr [100]. Could these state-of-the-art techniques assist in identifying ADP-ribosylated targets in the aging brain? Could it be that PARP-1, the most active and highly modified substrate known to date, is the substrate central to neurological disease characterized by abnormal ADP-ribosylation? If not, and if PARP-1 is simply one of many proteins that are abnormally modified in disease, how will ADP-ribosylated substrates crucial for brain function or specific to disease be identified?

Despite the need for a greater understanding of ADP-ribosylation function in the brain, initial studies in cells and animal models indicate that inhibition of PARP activity is a promising therapeutic for neurological disease, including ALS/FTD. Important advances have been made in developing rodent models of ALS/FTD that recapitulate features of the human disease [103–105]. Given the efficacy of PARP inhibition in rodent models of PD and ischemic stroke, and the reported benefit of PARP inhibition in neuronal models of ALS/FTD, PARP inhibitors may be of therapeutic benefit in ALS/FTD rodent models and beyond.

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