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Regulation of Biomolecular Condensates by Poly(ADP-ribose)

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Abstract

Biomolecular condensates are reversible compartments that form through a process called phase separation. Post-translational modifications like ADP-ribosylation can nucleate the formation of these condensates by accelerating the self-association of proteins. Poly(ADP-ribose) (PAR) chains are remarkably transient modifications with turnover rates on the order of minutes, yet they can be required for the formation of granules in response to oxidative stress, DNA damage, and other stimuli. Moreover, accumulation of PAR is linked with adverse phase transitions in neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. In this review, we provide a primer on how PAR is synthesized and regulated, the diverse structures and chemistries of ADP-ribosylation modifications, and protein–PAR interactions. We review substantial progress in recent efforts to determine the molecular mechanism of PAR-mediated phase separation, and we further delineate how inhibitors of PAR polymerases may be effective treatments for neurodegenerative pathologies. Finally, we highlight

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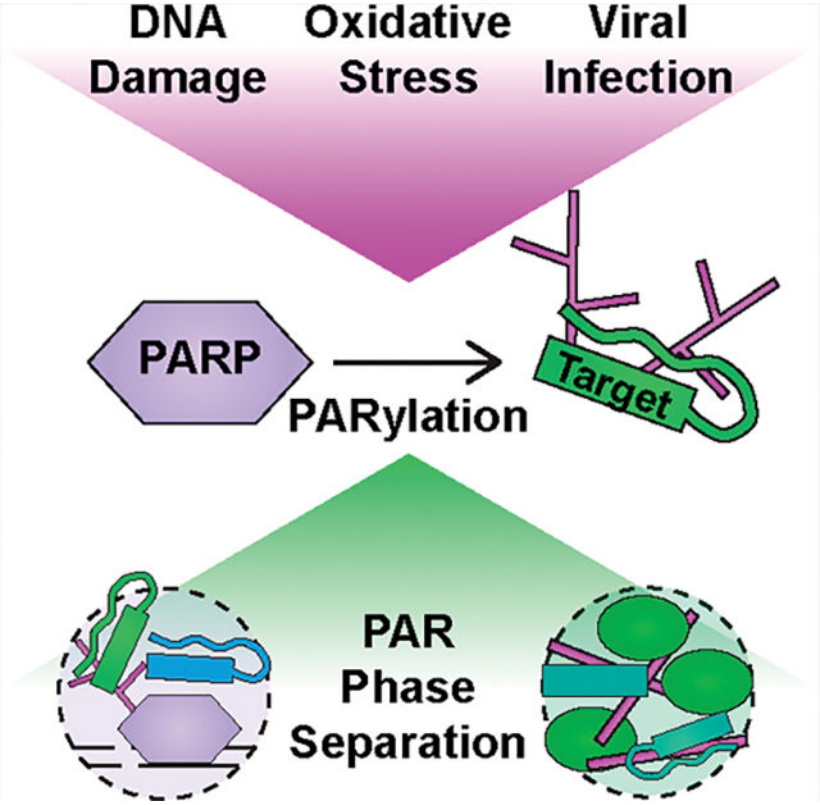
the need for rigorous biochemical interrogation of ADP-ribosylation in vivo and in vitro to clarify the exact pathway from PARylation to condensate formation.

Graphical Abstract



1. INTRODUCTION

The cellular response to various stresses relies upon the rapid and reversible recruitment of proteins, RNA, and other molecules into functional ribonucleoprotein (RNP) complexes.[1] Unlike membrane-bound organelles, the responding biomolecules are not compartmentalized by lipid bilayers, exposing the RNP complex to the surrounding cellular milieu. Instead, it is thought that RNP complexes undergo a phase transition into liquid-like granules, which are also called biomolecular condensates.[2] Proteins with intrinsically disordered regions (IDRs) and multivalent RNA molecules together promote this transition through a process called phase separation (PS), in which the dense RNP complex is a discrete phase with unique viscoelastic properties from the dilute phase.[3] Multivalent interactions allow RNP complexes to quickly form in response to cellular stimuli. PS may contribute to diverse biological processes such as the stress response, transcription, the DNA damage response, mRNA splicing, RNA degradation, and others.[4]

Two major challenges for the cell when assembling phase-separated compartments are (1) rapidly triggering PS in response to the external stimulus and (2) recruiting the correct biomolecules to the granule. Biomolecular condensates do not have a membrane that is

selectively permeable to specific proteins. Moreover, certain granules, like stress granules (SGs), must only assemble in response to acute stimuli, or cells cannot survive.[5,6] Therefore, the cell needs mechanisms to direct the formation of biomolecular condensates on demand. One emerging hypothesis is that a molecule called PAR enables such rapid organization of certain cellular condensates in species that express PARPs.[7–18]

Poly(ADP-ribose) (PAR) is a nucleic-acid-like polymer that is synthesized by poly(ADP-ribose) polymerases (PARPs).[19] PAR is added as a posttranslational modification to target proteins, where it can act as a signal for various biological processes. Unlike many other posttranslational modifications that deposit small chemical groups to certain amino acids,[20,21] PAR is a multivalent polymer that is synthesized directly on the protein. Therefore, PAR confers a unique biochemical property on the poly(ADP-ribosylated) (PARylated) protein: multivalency. In other words, a newly synthesized PAR chain can serve as a scaffold on which other proteins may assemble. Importantly, multivalency is a well-established universal mechanism to promote PS.[22]

PARP-dependent PARylation is best characterized in the DNA damage response.[17,18,23] Like other stress-related processes that we will describe in this review, PARPs rapidly synthesize PAR chains in response to DNA breaks (the stress), helping direct the recruitment of DNA repair proteins within minutes (the response). An emerging theme is that PAR can serve as a molecular trigger for DNA repair or potentially other stress responses, and as such, PAR can promote the formation of phase-separated granules at specific foci, like a DNA damage site.[17,18,24] Therefore, we propose that PAR-mediated interaction can serve as a unifying mechanism for initiating stimulus- or stress-induced granule formation. Such a mechanism has also been suggested by others.[7,9,11–14]

Here, we review recent advances in PS and PAR biology, focusing on how PAR drives the PS of diverse proteins in response to biological stress. First, we provide background information on PAR structure and synthesis. Next, we cover the covalent (i.e., posttranslational) and noncovalent binding of PAR to proteins, including the various protein domains that recognize PAR chains. With this primer, we then provide a detailed overview of the literature covering PAR's role in PS, including the DNA-damage response, stress granule formation and dissolution, viral infections, osmotic pressure sensing, and other roles. Finally, we link PAR PS to clinical studies showing increased PARylation and PARP activity in neurodegenerative diseases like Parkinson's disease, Alzheimer's disease, frontotemporal dementia, and amyotrophic lateral sclerosis. Further mechanistic studies with recent technical advances in PAR biology are needed to provide a more detailed understanding of PAR-mediated PS, but we hope that this review will provide the scientific foundation and impetus for these studies to occur.

2. POLY(ADP-RIBOSE) STRUCTURE AND SYNTHESIS

PAR is covalently attached to proteins by PARPs[19,25] (Table 1), and many PARPs are implicated in PS. PARPs use nicotinamide adenine dinucleotide (NAD^+) as a substrate for each ADP-ribose unit added to a target protein (Figure 1). Therefore, the structure of an ADP-ribose unit resembles NAD^+ without the nicotinamide group.[26,27] Target

proteins can be mono- or poly(ADP-ribosylated), and PAR chains can be up to ~200 units long.[28,29] Because PAR chains are covalently attached to proteins, long PAR modifications significantly impact the structure and biochemical properties of the target protein. PAR chains are also stiffer than RNA or DNA chains.[30] This section is a primer on the enzymatic cycle underlying PAR synthesis and catabolism, and the catalytic activity of PARPs.

2.1. The PARylation Cycle

Unlike many other posttranslational modifications, ADP-ribosylation marks are polymerized and depolymerized on proteins, allowing dynamic control of PAR chain length and structure. The canonical PARylation cycle starts with the deposition of mono-ADP-ribose units on proteins through a covalent linkage to the target protein.[40] Many PARPs can catalyze this initial mono-ADP-ribosylation (MARylation), and we discuss the covalent linkage of ADP-ribose to proteins in more detail below (see section ADP-Ribosylation of Proteins). The MARylation reaction uses a single NAD^+ molecule: the covalent linkage between the ribose sugar and the nicotinamide molecule is cleaved in a rate-limiting step, which allows the ribose sugar to be attached to the acceptor amino acid of the target protein. Many target proteins are MARylated under basal conditions;[41] stress events or other stimuli usually direct the PARylation reaction.[16]

Once a mono(ADP-ribose) (MAR) unit is added to the target protein, certain PARPs may further modify the protein to synthesize a polymerized ADP-ribose chain.[42] It is unclear whether MARylation added by one PARP may act as a substrate for PARylation by other PARPs. The formation of a 2′–1′′ ribose–ribose glycosidic bond underlies the PARylation reaction, which can be sequentially catalyzed on each terminal ADP-ribose unit.[43] Every ADP-ribose unit requires a new NAD^+ molecule.[42] PAR chains range in length from ~2–200 units,[28,29] meaning that PAR chains act as an NAD^+ sink during extensive PARylation events.[44] Moreover, branching of the PAR chain may be initiated by PARP1/2; here, the PARP catalyzes the formation of a 2″–1′′ ribose–ribose bond in addition to the usual 2′–1′′ linkage.[27,45] PAR branching is spaced every ~20–50 units,[26,28] allowing further PARylation at new terminal ADP-ribose units. Branching allows highly PARylated targets to adopt a “starfish” morphology with huge PAR chains emanating from a single initiating chain.[46] For more on the structural heterogeneity of PAR chains, we refer the reader to a recent review in ref 47.

Depolymerization of PAR is mediated by PAR glycohydrolases (PARGs), which act through endo- or exoglycosidic cleavage of PAR chains (Table 2).[48] Exoglycosidic cleavage is generally more common, meaning that individual ADP-ribose units are typically released as the PAR chain is depolymerized from the end of the modification.[49] Certain PARGs may release intact PAR chains via endoglycosidic cleavage,[50] but free PAR is readily catabolized by basal expression of PARGs in cells. Release of the initial ADP-ribose unit (i.e., MARylation) is mediated by specific PARGs that recognize the unique protein-ADP-ribose linkage.[51] Thus, dePARylation and deMARylation are functionally decoupled and usually occur independently.

Therefore, a dynamic cycle of MARylation, PARylation, dePARylation, and deMARylation defines the PAR cycle. Each of these steps regulates the recruitment of proteins to PAR foci and the formation of phase-separated condensates.

2.2. Mechanism of PAR Chain Synthesis

The canonical PARP active site consists of a histidine–tyrosine–glutamate (H–Y–E) triad, which is essential for polymerization (Figure 2). All PARylating PARP enzymes contain the H–Y–E triad.[52] However, the H–Y–E-containing PARP3 and PARP4 are unable to synthesize PAR chains, indicating that the triad is not sufficient on its own for PARylation.[52] Natural variations of the H–Y–E triad in other PARPs (e.g., H–Y–I, H–Y–L, etc.) can still engage in MARylating activity.[52] Many structural studies use PARP1, the main nuclear PARP enzyme, and the founding member of the PARP family, as their model, but homology between PARP1 and other PARylating PARPs implies that many of the catalytic activities are similar.[53,54] For a more complete review on PARP1 synthesis of PAR chains, we refer the reader to ref 55.

In PARP1, triad amino acids His-862 and Tyr-896 are required for NAD^+ binding.[56] His-862 contacts the 2′-OH of the adenosine–ribose of NAD^+, and Tyr-896 stacks with the nicotinamide ring.[56] These two residues are essential for proper catalysis: the PARPs without His-862 and Tyr-896 equiv, PARP9 (Q-Y-T) and PARP13 (Y–Y–V), are either weakly active or completely inactive, respectively.[52,57] By contrast, the last triad residue Glu-988 is required for destabilizing NAD^+ and covalently attaching the remaining ADP-ribose molecule to the target protein or ADP-ribose.[43] Glu-988 performs this activity by hydrogen bonding with the 2′-OH of the nicotinamide ribose, which allows the target protein side chain to perform a nucleophilic attack on the ribose–nicotinamide bond.[43] Glu-988 primarily serves to position NAD^+ and the acceptor site in the correct orientation.[43,58] Other structural elements, such as the donor and acceptor loops, further modulate the catalytic activity of PARP enzymes.[52,59] Mutations at nearby residues impact PAR branching efficiency of PARP1.[45,60] Finally, accessory factors may help terminate PARylation reactions, shifting the PAR cycle toward dePARylation.[61] Before dePARylation occurs, many proteins may noncovalently bind to the covalently bound PARylated protein, as we discuss in the next section.

3. PROTEIN-POLY(ADP-RIBOSE) BINDING

Poly(ADP-ribose) chains are added as a posttranslational modifications to proteins. Therefore, there are two main modes of protein–PAR binding: (1) covalent attachment of the ADP-ribosylation modification to the target protein and (2) noncovalent binding of the PAR chain to a PAR-binding protein (Figure 3). Together, these two interactions provide exquisite specificity; PAR chains can be synthesized on certain proteins in response to stimuli, which then recruit binding partners to the new PAR chains. PARG and PARG-like enzymes disrupt both interactions by degrading the PAR chain from the target protein. Given the remarkably transient nature of ADP-ribosylation, the covalent and noncovalent PAR interactions can be brief, only occurring when the correct biological stimulus promotes PAR synthesis.

In the context of PS, the synthesis of the PAR chain provides a multivalent scaffold for the assembly of PAR-binding proteins on the target protein. A naked, newly synthesized PAR chain can be recognized by proteins with PAR-binding domains, also known as PAR readers. If the PAR chain is of a sufficient length, multiple PAR readers can assemble on a single target protein, which then promotes a phase transition. In this model, the PARylated protein is recruited into the phase-separated condensate, even if it cannot undergo PS on its own. An alternative mechanism is that PAR can induce conformational changes in proteins by freeing IDRs, which then promote PS, perhaps independently of PAR.

This section will review the biochemistry underlying the ADP-ribosylation modification and recognition of the PAR modification by other proteins. In particular, we will focus on how PARPs target certain proteins for ADP-ribosylation, which amino acids accept ADP-ribosylation modifications, and cofactors that may alter ADP-ribosylation activity of PARP enzymes. Then, we will discuss various domains that interact with PAR chains, the enrichment of PAR-binding domains in disordered regions of proteins, and how PAR binding aligns with other binding interactions.

3.1. ADP-Ribosylation of Proteins

ADP-ribosylation modifications are added by PARPs, as discussed in section Poly(ADP-ribose) Structure and Synthesis above. There are 17 PARP enzymes in humans, of which 16 are catalytically active[52] (Table 1). Only 4 of the 16 PARP enzymes can synthesize PAR chains: PARP1, PARP2, PARP5a, and PARP5b.[52] The first two enzymes, PARP1 and PARP2, are predominantly nuclear, though PARP2 has been identified in puncta in the cytoplasm.[62] PARP2 mediates branching of PARP1-synthesized PAR chains.[33] PARP5a and PARP5b have high sequence similarity,[63] and they are localized to the cytoplasm.[16,62] PARP5a and PARP5b cannot synthesize branched PAR chains,[64] so it is thought that most cytoplasmic PAR modifications are linear chains.

The remaining 12 PARPs only add mono(ADP-ribosylation) modifications. The exact interplay between MARylating PARP enzymes and the PARylating PARP enzymes is unknown; MARylating PARPs may target unique proteins for ADP-ribosylation, which could then be targeted for PARylation by other PARPs, although the evidence for this theory is lacking.[65,66] Certain MARylating PARPs may also modulate the PARylation activity of other PARPs. For example, PARP3 can stimulate PARP1 activity in the absence of DNA.[67] Because ADP-ribosylation-mediated PS appears to rely on the multivalency of PAR chains (see section Molecular Interactions Underlying PAR-Mediated Phase Separation below), we will focus our discussion on PARylating PARPs.

PARP1 and PARP2 synthesize the vast majority of PAR in cells,[68] and they predominantly initiate ADP-ribosylation at serine residues.[69–74] Serine targeting is mediated by the cofactor histone PARylation factor 1 (HPF1). Structural studies of PARP1-HPF1 binding demonstrate that HPF1 completes the active site of PARP1, biasing PARP1 toward serine ADP-ribosylation.[61] HPF1 also sterically hinders automodification of PARP1, and HPF1 binding blunts the length of PAR chains synthesized by PARP1.[61,72,75] The exact motif targeted by the PARP1/2-HPF1 complex is unknown, although likely involves nearby basic residues.[70,76] The ADPriboDB tool maintains a list of ADP-ribosylated targets.[77]

Notably, PARPs are themselves major targets of ADP-ribosylation through automodification reactions.[78]

ADP-ribosylation may also occur at other residues, including arginine,[40,74,79–95] aspartate,[52,96] cysteine,[52,82,97–99] glutamate,[52,96] histidine,[74] lysine,[52,100] and tyrosine.[74,101] PARPs appear to have different preferred targets; for example, PARP8 may prefer modifying cysteine residues.[52] Therefore, MARylating PARPs may target proteins or sites that are otherwise not recognized or efficiently modified by the PARylating PARP enzymes.

PARP activity is promoted by environmental stimuli such as oxidative stress and DNA-damaging agents.[16,34,102] These perturbations activate biological responses that require the activity of PARP1, PARP5a, or both, but the exact mechanism of how ADP-ribosylation activity increases in response to this stimulus is unclear. PARP1/2 activity is directly stimulated by DNA damage, which is recognized by DNA-binding domains of PARP1/2.[103] Notably, the surge in ADP-ribosylation mediated by environmental stress is fast, increases in PAR levels can be detected within minutes.[60] Recent studies have indicated that PARP activation is upstream of stress-mediated PS,[17,18,34] so the exact molecular mechanism of how ADP-ribosylation is stimulated by stress should be of intense interest to the field.

3.2. Free PAR Chains

PARPs require a protein target for their ADP-ribosylation activity, so free PAR is not directly synthesized by PARPs. However, dePARylating enzymes like PARG can release free PAR chains via endoglycosidic cleavage of the PAR chain on an ADP-ribosylated protein (Figure 4).[48–50,104–106] The preferred enzymatic activity of PARG is exoglycosidic cleavage, but endoglycosidic cleavage occurs in ~20% of cleavage events.[49] TARG1 can also release free PAR by cleaving the ADP-ribosylation linkage.[51]

The basal expression of PARG and ARH3, which also has robust exoglycosidic activity, suggests that free PAR is rapidly degraded.[107–111] Indeed, H_2O_2-stimulated PAR chains were observed to rapidly degrade within 20 min of oxidative stress.[60] Branched PAR chains may be more resistant to PARG/ARH3 activity.[60] Despite the widespread use of purified PAR chains in many biochemical studies, direct evidence of appreciable free PAR in cells is limited.

Evidence for free PAR chains primarily comes from nuclear PARP1 exerting influence over cytoplasmic biological processes; some studies have suggested that PARP1 activity may regulate localization of cytoplasmic PAR-binding proteins. For instance, PARP1 activity mediates the translocation of apoptosis inducing factor 1 (AIF1) from the mitochondria to the nucleus.[112,113] PARP1 regulates the localization of predominantly nuclear proteins like TAR DNA-binding protein 43 (TDP-43) and hnRNPA1 to cytoplasmic stress granules.[31,114] Indeed, PARP1 inhibitors promote nuclear retention of TDP-43 and prevents formation of cytoplasmic TDP-43 aggregates.[114] It is possible that TDP-43 and hnRNPA1 are ADP-ribosylated in the nucleus and exported to the cytoplasm. Other studies have suggested that PARP1 has little role regulating G3BP1 or FUS localization to cytoplasmic stress granules.[34] Given that PARP1 is a predominantly nuclear protein, its PARylation activity should be limited to the nucleus; data suggesting that it exerts an effect on cytoplasmic

PAR-binding proteins may imply the release of free PAR that is exported to the cytoplasm. Further studies are needed to clarify whether free PAR can be stably maintained in cells without inhibiting PARG activity.

3.3. PAR Readers

Noncovalent interactions with PAR chains are mediated by a variety of protein domains, including monofunctional domains that only bind PAR and multifunctional domains that engage with other binding partners (for a more comprehensive review of the subject, please see ref 10). PAR readers recognize PAR chains through the diverse functional groups on the ADP-ribose polymer, including the adenosine base (PAR binding zinc fingers), the *iso*-ADP-ribose linkage (WWE domains), and the entire ADP-ribose unit (Macro domains). Other domains like RGG repeats engage in more nonspecific interactions with the highly negatively charged backbone. Therefore, even proteins without defined PAR-binding domains may interact with PAR chains. This section will give an overview of identified PAR-reader domains (Figure 5).

The most common PAR-reader domain is the PAR-binding motif (PBM), which was identified in a proteomics study of PAR-binding proteins.[115] Recognition of PAR is mediated by a mixture of basic and hydrophobic residues stretching ~20 amino acids: $[HKR]_1$-X_2-X_3-$[AIQVY]_4$-$[KR]_5$-$[KR]_6$-$[AILV]_7$-$[FILPV]_8$. The trio of KR motifs are the most important constituents of the PBM because they likely recognize the negatively charged PAR backbone.[116] The strong electrostatic attraction allows some PBMs to achieve affinities in the nanomolar range.[117] Multiple PBM regions can contribute to a multivalent protein–PAR interaction; for instance, the ALS-associated TDP-43 has two distinct PBMs embedded in its nuclear-localization sequence (NLS), which together promote strong association with PAR chains in vitro and in vivo.[118,119] Notably, many hnRNP proteins, which undergo PS, contain PBMs, but these do not always appear in the NLS.[120]

WWE domains, consisting of a pair of conserved tryptophan residues and glutamate, are found in PARPs and ubiquitin ligases.[121] The WWE pocket binds to the *iso*-ADP-ribose linkage (i.e., the ribose–ribose sugar linkage in a PAR chain) with micromolar affinity,[122–126] but it has much weaker binding to the monomeric ADP-ribose unit. Therefore, WWE proteins mostly recognize poly(ADP-ribosylation), not mono-(ADP-ribosylation). WWE domains enable certain MARylating PARPs (e.g., PARP11, PARP12, and PARP14) to bind PAR chains, recruiting them to PAR foci. As discussed below, PARP12 translocation from the Golgi to the stress granule relies upon its WWE domain interacting with PAR chains synthesized upon oxidative stress.[37] WWE domains are also found in several E3 ubiquitin ligases, suggesting a functional connection between PARylation and ubiquitination, perhaps to target PAR-binding proteins for degradation.[122,127–130] Indeed, PARP1, which itself is one of the main targets of PARylation in cells, is targeted for degradation when it is autoPARylated, and the WWE-containing E3 ubiquitin ligases Iduna and TRIP12 mediate this action.[131,132]

Macro domains are also present in PAR metabolic enzymes, including PARP9, PARP14, PARP15, PARG, TARG1, MacroD1, and MacroD2.[51,104,133–135] The Macro domain is a conserved ~100–200 amino acid domain with nanomolar affinity for PAR chains.[134,136]

Unlike other PAR readers, Macro domains only recognize the terminal ADP-ribose unit. Macro domains are found in many viral proteins, including the nsP3 protein of SARS-CoV-2, and are often paired with glycohydrolase activity.[133,137–140] As discussed below, viral Macro domain-linked glycohydrolase activity is linked with turnover of stress granules.[133] Interestingly, some histone variants also contain Macro domains, and these Macro domains can help localize histone variants and chromatin remodelers to regions with PARP1 activity, i.e., double-stranded DNA breaks (DSBs).[141–143]

Some DNA damage response proteins contain a modified zinc finger that binds PAR molecules: PAR binding zinc fingers (PBZ).[144–151] The PBZ domain consists of a conserved amino acid motif that resembles canonical zinc fingers:[145] [K/R]xxCx[F/Y]GxxCxbbxxxxHxxx[F/Y]xH. Recognition of PAR chains by the PBZ domain hinges on adenine bases.[145] The specificity of PBZ for PAR chains allows efficient recruitment of diverse DNA damage response proteins like Ku, Chk2, RAD17, APLF, CHFR, and others. As with other zinc fingers, PBZ requires zinc for nanomolar affinity to its binding partner.

RNA-recognition motifs and other nucleic acid binding domains may also recognize PAR, albeit with lower affinity than for their preferred substrate.[117,152–155] This bifurcated binding ability leads to a competitive interaction between the protein, PAR, and DNA/RNA, which can tune the biophysical properties of condensates or regulate the biological function of the protein–PAR interaction.[24,34]

Finally, some of the most highly enriched PAR readers do not contain a canonical PAR-binding domain per se; instead, they have repeats of positively charged residues, such as RGG repeats, KR-rich motifs, or SR repeats.[102,156,157] Like the PAR-binding motif, the positively charged arginine residues contribute to a strong electrostatic interaction with negatively charged PAR chains. For example, the arginine residues of FUS, which are clustered in three RGG repeats, are required for localization of FUS to DNA damage foci and to stress granules.[17,24,34]

Importantly, RGG domains can independently promote PS, and the toxic dipeptide repeat protein, poly(GR), is linked with neurodegeneration in c9ALS/FTD.[158–161] Because both PAR binding and PS propensity are encoded within RGG repeats, the two biochemical interactions may regulate each other. PAR binding may prevent or promote individual RGG domains from interacting with other disordered regions, inhibiting or promoting PS, respectively. For example, PAR associates with poly(GR) in vitro and in post-mortem brain tissue, and appears to promote poly(GR) condensation, suggesting a role of PAR in promoting dipeptide repeat toxicity in c9ALS/FTD.[162] Furthermore, tandem RGG domains, such as those observed in FET family proteins, can coordinate PS by binding PAR with some RGG repeats and other proteins with other RGG repeats. Indeed, proteins with tri-RGG domains are particularly enriched among PAR readers.[156]

More broadly, the other types of proteins that contain PAR-binding domains also skew toward phase-separation-related processes. Several recent studies using proteomics-based approaches identified and quantified the relative binding of PAR readers to ADP-ribosylated proteins.[102,115,156,163] RNA-binding proteins, RNA helicases, and RGG-containing proteins

were among the most enriched PAR readers.[156] Many of these proteins undergo, regulate, or are implicated in PS events.[3,164–173] PAR readers also tend to be enriched in biological processes that are thought to involve PS, including DNA repair, RNA splicing, glycolysis, and translation.[17,168,174–176] Therefore, there is a strong link between noncovalent protein–PAR interactions and PS.

4. POLY(ADP-RIBOSE)-MEDIATED PHASE SEPARATION (PS)

Membrane-bound organelles are surrounded by lipid bilayers that confer several advantages: first, they allow cells to compartmentalize various reactions; second, they protect or sequester certain proteins and nucleic acids through their semipermeable membranes; third, organelles have carefully controlled internal environments. However, canonical organelles are inefficient at responding to external stimuli, and the cell expends a lot of energy to maintain their specialized environments. For instance, the cell must establish and sustain a Ran-GTP/-GDP gradient to direct nucleocytoplasmic transport.[177]

Membraneless granules circumvent these shortcomings by using the physical properties of PS to reversibly generate dynamic compartments. Granules are not protected by a membrane, so constituent biomolecules can readily diffuse in and out. They are also more easily dissolved by enzymes or changes in cellular salt concentrations. However, the dynamism of membraneless granules allows the cell to respond to stress or damage by quickly compartmentalizing proteins, RNAs, and other molecules.[178]

PAR is uniquely positioned to support PS in PARP-expressing cells. Because PAR chains are readily synthesized and then rapidly degraded, they can direct the formation of phase-separated granules and assist with the dissolution of granules, too. The chemical nature of the PAR chain also potently promotes PS: it is a negatively charged multivalent polymer able to bind many PAR readers at once. As previous reviews have noted,[7,9–14] PAR is involved in several biological processes that are associated with PS (Figure 6). In this section, we will discuss the biophysics of PS, the mechanisms of protein–PAR PS and review the literature that describes the role of PAR in biomolecular condensates.

4.1. The Biophysical Principles of Phase Separation

Phase separation (PS) occurs when it is more energetically favorable for multivalent polymers to coalesce into a dense condensate within a dilute liquid phase. The coexistence of two phases is the hallmark of a phase-separated system. When the condensate is a liquid phase, it is formally referred to as a coacervate, and a coacervate usually consists of biological polymers like polypeptides and nucleic acids.[179] Coacervation occurs when the dense liquid phase exists in thermodynamic equilibrium with the surrounding dilute phase, and the coacervation thermodynamics can be described by the Flory–Huggins model[180] (see ref 181 for a review on the subject). Biological coacervates often form via associative interactions between biopolymers, which is a type of coacervation called complex coacervation.[182] Importantly, the dilute phase retains some of the molecules that are concentrated within the complex coacervate; in a biological context, this means that a significant fraction of proteins or RNAs that are concentrated within a granule also exists in the cytoplasm or nucleoplasm.[183]

A key element of complex coacervation is the associative interactions between biopolymers. In practical terms, one molecule may act as a scaffold, which recruits clients into the coacervate.[22] The valency of the scaffold is a critical part of the associative polymer model: if a scaffold can accommodate many clients, it can increase the local concentration of the of the biopolymers into the dense phase.[3,184] The network that arises from these interactions drives the formation of the coacervate, thereby causing PS. The conditions that support PS can be clearly delineated in a phase diagram, in which the concentration of the dense biopolymer is usually plotted versus changes in another environmental factor.[185] The coexistence line on the phase diagram denotes the transition from the one-phase system to the two-phase system. Crossing the coexistence line begins the nucleation process, allowing the formation of new condensates. Recent reports have indicated that nucleation may also initiate in the one-phase system.[186,187]

Importantly, biological PS is often triggered by changes in the concentration of biopolymers like the release of mRNA during stress or the translocation of proteins from one region of the cell to another. Environmental changes can also mediate PS, including shifts in pH, salt concentration, temperature, or pressure. Such changes may lead to reentrant phase transitions, in which the two-phase system devolves back into a single-phase, well-mixed system. This may occur if the valency of the scaffold is too high, which will disperse the client to such an extent that it cannot form a dense coacervate.[173]

4.2. Phase Separation in Biology

In biology, one of the first descriptions of PS was the P granule in *Caenorhabditis elegans*.[169] Many groups have since reported biological PS for a variety of cellular granules, including stress granules,[188,189] P bodies,[190,191] TIS granules,[192,193] G bodies,[174,194] the nucleolus,[195,196] paraspeckles,[197,198] histone locus bodies,[199] DNA repair granules,[17,18] and others.[4] In cells, phase-separated condensates are generally called granules; in vitro condensates are usually referred to as droplets. Condensate is a generic term to refer to biomolecular structures that does not presuppose the material state of the structure. Other terms, such as aggregate or amyloid, describe solid-like condensates that adopt distinct structural patterns. By contrast, liquid-like condensates (i.e., coacervates) demonstrate wetting, fusion, and other characteristics reflective of true liquids, and these parameters can be quantified by physical characteristics like viscosity and elasticity (for a review of the liquid properties of condensates, please see ref 200 and in addition, ref 201 discusses the differences between liquid–liquid PS and PS in more detail). Liquid-like granules can mature into gel-like or solid-like condensates through a process termed percolation,[202,203] which may contribute to disease pathology (see section Accumulation of Poly(ADP-ribose) in Neurodegenerative Pathologies below). In this review, we generally refer to any phase-separated body as a condensate or granule so that we do not presume the material properties of the condensate. Granules have been proposed to accelerate enzymatic reactions, concentrate biomolecules, buffer the internal environment, sense environmental changes, among other roles.[185] Given the ubiquitous presence of phase-separated granules in the cytoplasm and nucleus, there is intense interest in understanding the regulation, function, and dissolution of condensates.

Multivalent interactions between biopolymers drive the formation of the dense condensate phase (for a thorough review of the physical processes underlying PS, please see ref 181). The associative polymer model posits that proteins and other biomolecules are composed of so-called "sticker" and "spacer" regions,[166,204] which together determine the relevant parameters of PS, including the protein concentration at which PS occurs (C_{sat}). Stickers are regions of the polymer that can associate with other polymers; examples include residues that form cation–π and π–π interactions, like arginine or tyrosine, and domains that promote multivalent binding interactions like RNA-binding domains or PAR readers.[22,205] Meanwhile, spacers are the residues or domains that do *not* participate in PS although they may control percolation.[206,207] By definition, all regions that are not stickers are spacers and vice versa. Not all stickers are equally strong at promoting PS, though; for instance, lysine is a weaker sticker than arginine.[166]

What determines whether a protein may undergo PS? In general, the presence of enough sticker regions to promote multivalent assembly of a dense phase is required. Proteins with certain amino acids tend to self-associate and multimerize into condensates. For example, arginine and tyrosine can promote PS,[166,204] and other charged residues also support PS by electrostatic interactions.[208] IDRs may also engage in multivalent interactions that drive PS (for a review on IDRs, see ref 209). Prediction software like IUPred help determine whether a protein is disordered or not.[210,211] As mentioned above, binding domains can also function as stickers by promoting multivalent interactions.

4.3. Molecular Interactions Underlying PAR-Mediated Phase Separation

The associative polymer model helps explain why biomolecules like PAR can promote PS. If we consider PAR readers to be sticker domains, then PS propensity is directly correlated to the number of PAR readers and PAR chains present in the system. PAR chains may also act as stickers, in which a minimal PAR length (n) is sufficient for protein binding and each multiple of this minimal requirement ($2n$, $3n$, etc.) increases the multivalency of the protein–PAR interaction. Therefore, PAR chains will directly increase the PS propensity and decrease the observed C_{sat}, a phenomenon that has been observed in vitro.[31,34,118] Consistent with this observation, mono(ADP-ribose) is usually insufficient to promote PS.[31,34,118]

The minimal PAR chain length required for protein binding depends on the PAR reader. The tumor suppressor protein p53 can form monomers with 16-mer PAR but requires longer PARs of >40 units for stronger, multimeric binding.[212] A similar dependence of 40+-unit PAR was observed for the oncoprotein DEK.[213] Biological processes mediated by PAR chains such as the parthanatos cell death pathway and inhibition of cell cycle progression via activation of Chk1 are also promoted by longer PAR chains of >40 units.[112,113,146] Likewise, PARP1 binding increases with longer PAR chains,[156] which may provide a positive feedback loop to promote robust and rapid formation of PAR chains. Some proteins, such as NONO, XRCC1, and PARG, appear to bind shorter PAR chains with higher affinity.[156]

A recent study examining FUS condensation with PAR more directly linked PAR length with PS.[34] FUS multimerization increased as a function of PAR length, and PAR chains

of 16 units or longer enabled the formation of FUS multimers.[34] The apparent binding affinity of FUS for PAR also increased by ~20-fold for 32-mer PAR compared to 8-mer PAR.[34] Increased PAR binding directly correlated with increased PS in vitro,[34] indicating that longer PARs more strongly promote condensation of PAR readers. Other studies have likewise shown FUS PS in response to DNA damage-mediated PARP1 activity, which forms long PAR chains of >30-mer.[17,18,24] Therefore, the multivalent scaffolding afforded by a long PAR chain supports PS, a similar phenomenon to what has been observed with RNA.[214–217] PARylation of multiple sites on the same protein may also achieve multivalency.

Less is known about the effect of PAR branching on PS. The branching of PAR chains may be considered analogous to secondary structures like hairpins and stem loops in RNA molecules, which affect the affinity of proteins for RNA.[218–220] Indeed, a few studies have shown that certain PAR readers may prefer branched PAR chains.[123,221,222] Branched PAR modifications may increase PS through a few distinct mechanisms: (1) incorporation of new proteins that otherwise would not easily interact with linear PAR, (2) added multivalency by increasing the local concentration of minimal PAR chains (n), or (3) increasing the stability of condensates through a more complex binding network. In line with the last hypothetical, branched PAR chains likely impact the material properties of PAR-mediated condensates by forcing PAR readers into unique conformations or more highly concentrated oligomers. It is important to note that branched PAR chains are only formed by nuclear PARPs,[64] indicating that branched PARylation likely is not a major factor in cytoplasmic PS. However, there are some instances in which PARP1 is mislocalized to the cytoplasm,[223] and it is also possible that branched PAR on target proteins may be exported.[224]

Other posttranslational modifications also regulate PS.[20,21,225,226] For instance, arginine methylation can reduce PS by dampening sticker contacts of arginine residues or binding to RNA.[227–233] However, arginine methylation of TDP-43 allows PS but disfavors pathological aggregation.[233] Phosphorylation of serine and threonine residues can either inhibit or promote phase transitions.[232–238] By contrast, PARylation of proteins usually promotes PS, likely because PARylation introduces a new scaffold for PAR-reader binding and multimerization. Instead of modifying the biochemical properties of existing stickers like arginines, PARylation provides creates a multivalent sticker, enabling quick and reversible formation of condensates. We do note that very high concentrations of PAR chains may buffer PS by diluting the multivalency of protein–PAR binding networks.[34,173]

Therefore, given the transient nature of PARylation and its inclination to promote PS, several biological processes appear to rely on PAR chains for efficient condensation. The following sections will discuss biological examples of PAR-mediated PS in more detail.

4.4. The DNA Damage Response Requires Phase Separation of PAR Readers

The role of poly(ADP-ribose) and PARP1 in the DNA damage response is well established. PARP1 activity is essential for the identification of single- and double-stranded breaks, recruitment of DNA damage repair proteins, and resolution of the DNA lesion (for a comprehensive review of PARP1 in the DNA damage response, we refer the reader to ref 239). Poly(ADP-ribosylation) modifications are rapidly added to histones, DNA, and

PARP1 itself.[73,240–242] Single- or double-stranded breaks are required for PARP1-mediated synthesis of PAR chains.[23,103,243–246] In fact, increased PARP1 activity is often observed in cancer;[223,247–249] enhanced PARP1 expression is needed so that the higher rate of DNA damage can be addressed, but PARP1 activation also upregulates other inflammation-related and oncogenic factors and can initiate error-prone DNA damage repair pathways.[250–252] Small-molecule inhibitors of PARP1 activity are approved for clinical use with certain cancers.[253–255] PARP1 acts upstream of both nonhomologous end-joining (NHEJ) and homologous recombination (HR),[239] highlighting its essentiality in resolving DSBs.

An important finding in the field was the formation of PARP1-dependent phase-separated compartments in the DNA damage response (Figure 7).[17] PAR synthesis by PARP1 is rapid (occurring on the order of seconds), and turnover of PAR chains is equally quick (within minutes).[256] The PS-prone FET family proteins are recruited shortly after PAR synthesis (within seconds to minutes), strongly interacting with PAR.[17,18,24,257,258] The FET family consists of three related tri-RGG proteins: FUS, EWSR1, and TAF15.[259] Each of these proteins can form droplets in vitro,[18,166,260] and PS characteristics were observed at the DNA damage foci to which FET family proteins are adsorbed.[17,24] The RGG domains of FET proteins are critically important for this association with the DNA damage site.[17,24] Moreover, the prion-like domain of FUS is also required for DNA repair initiation.[261] The individual FET family proteins appear to direct the formation of the phase-separated DNA damage compartment.

PAR-mediated FUS recruitment is required for proper resolution of the DNA damage site.[261] PAR chains robustly promote the formation of FUS condensates,[17,18,34] and FUS recruitment to the DNA damage site is PARP1-activity dependent.[24] Loss of FUS significantly delays recruitment of proteins required for the DNA damage response, including 53BP1, NBS1, Ku80, and SFPQ.[261] Importantly, disruption of these FUS interactions leads to cytoplasmic mislocalization of FUS and subsequent neurodegenerative phenotypes.[262,263] The formation of the γH2AX histone variant is also dependent on FUS.[261] Transcriptional-associated DNA damage resolution may also require FUS.[264] Therefore, it is reasonable to hypothesize that PAR-mediated FUS PS is essential for proper progression of the DNA damage response.

Although several models have been proposed for PARP1 ejection from DNA damage sites following repair,[265] recent evidence suggests that EWSR1 binding is required for efficient PARP1 displacement.[266] Depletion of EWSR1 leads to hyperaccumulation of PARP1 at DNA damage foci,[266] indicating that the DNA damage response is stalled. It is also possible that EWSR1 is essential for the recruitment of other proteins that eventually eject PARP1. The role of the final FET protein family member, TAF15, in the DNA damage response is not known. Following ejection from the DNA damage site, PARylated PARP1 is targeted for proteasomal degradation by the WWE domains of the E3 ubiquitin ligases Iduna and TRIP12.[131,132]

Other RG- and IDR-containing proteins likely contribute to PAR-mediated PS. A recent study identified that the splicing factor USP39 directs NHEJ in response to PARP1 activity.[267] Like FET proteins, USP39 PS is RG-motif dependent.[267] Recruitment of

XRCC4, LIG4, APTX, and PAXX, all of which are required for NHEJ, follows USP39-PAR PS.[267] Excessive recruitment of USP39 may eventually downregulate HR by depleting BRCA mRNA through its role in the spliceosome.[267,268]

Moreover, PAR binding and PS have been observed in a decoupled manner for other proteins. For example, p53 is known to oligomerize on PAR chains,[212] p53 can be PARylated,[269] and PS of p53 was recently described in vitro and in vivo.[270,271] Thus, PAR may mediate condensation of additional proteins, perhaps working in tandem with the highly PS-prone FET family proteins. PARP1 activity is also enriched in the phase-separated nucleolus,[272] where it regulates ribosome biogenesis and DDX21 activity.[152,273] PARylation-independent PS also contributes to the DNA damage response through the protein 53BP1, which is recruited to damage foci independently of PARP1 activity.[274,275]

A recent report suggests that PARylation plays a role in antagonizing transcription, especially in response to DNA damage at the transcriptional locus (Figure 8).[32] If PARP1 senses DNA damage at a transcriptional locus, it PARylates the elongation factor P-TEFb.[32] Importantly, PARylation of P-TEFb inhibits its PS.[32] Although PAR chains usually promote PS, PARylation of P-TEFb neutralizes the self-association of nearby positively charged P-TEFb residues, indicating that the effect of PAR on PS is context-dependent.[32] This disruption prevents P-TEFb from hyperphosphorylating RNAP II, which is required for elongation of mRNA.[276] Other reports indicate that PARylation regulates transcription,[277–282] and it is hypothesized that PS augments transcriptional activity.[283–287] Therefore, an interplay between transcriptional PS and the DNA damage response PS may exist in which the factors involved in each process are mutually exclusive.

The exact spatiotemporal relationship between the various proteins contributing to PAR-dependent PS at DNA damage foci is unclear. It is likely that the synergistic effect of many PAR readers with PS-prone domains (e.g., prion-like domains of FET proteins) contributes to the formation of a dynamic,[288] reversible DNA damage compartment that is a bona fide phase-separated granule. In addition, the phase-separated DNA damage foci may also direct exactly which type of DNA repair occurs at double-stranded breaks: NHEJ or HR. It is possible that PAR chain structural heterogeneity (i.e., branched versus linear, short versus long chains) encodes regulatory input for the DNA damage response. Nevertheless, an abundance of evidence supports the notion that the phase-separated DNA damage response is seeded by PAR chains in a PARP1 activity-dependent manner.

4.5. Stress Granules Are Nucleated by PAR Readers and PAR Chains

Stress granules are cytoplasmic phase-separated condensates that form in response to environmental stressors, such as temperature changes or the presence of oxidative agents.[289] RNAs and IDR-containing proteins contribute to the rapid formation of stress granules,[215,290] and it is thought that stress granules protect certain mRNAs from degradation until the stress event recedes. A pair of related IDR-containing proteins are required for stress granule formation: G3BP1 and G3BP2.[167,234,291] In addition, multiple PARPs localize to stress granules in PARP-expressing cells, including PARP5a, PARP12, PARP13, PARP14, and PARP15.[16] PAR is also enriched within stress granules,[34] although stress granule PARG enzymes may counteract some PARP activity.[16] PAR chains readily

interact with many stress granule components, including G3BP1, hnRNPA1, TDP-43, and FUS (for a more extensive review on PAR in stress granules, we refer the reader to ref 292). Moreover, PAR production is stimulated by some of the same stresses that promote stress granule formation.[34]

Recent studies have suggested that PAR synthesis is required for the localization of IDR-containing proteins to stress granules (Figure 9a). G3BP binding to PAR is necessary for stress granule formation under most conditions.[102] The PBMs of the stress granule protein TDP-43 lie within its NLS.[118] This finding indicates that TDP-43 localization may be differentially regulated by competition in binding to the NLS between PAR and nuclear-import receptors.[118,293–296] In fact, interactions between PAR and TDP-43 are required to solubilize and effectively localize TDP-43 into stress granules;[118,297] moreover, PAR binding to TDP-43 through its PBM antagonizes neurodegeneration-linked TDP-43 aggregation.[114,119,298] Likewise, hnRNPA1 localization to stress granules is promoted by PARylation and PAR binding of hnRNPA1, which also promote co-condensation with TDP-43.[31] TIA-1 and other stress granule proteins are likely PARylation targets.[16] FUS recruitment to stress granules is dependent on PARP5a-mediated PAR synthesis, and PAR likely interacts with the arginines in the RGG domains of FUS.[24,34]

However, one major question is the source of PAR that is localized in the stress granule. An obvious choice would be the stress granule-associated PARPs, especially the PARylating enzymes PARP5a and PARP5b, which also interact with TDP-43 via its tankyrase-binding motif in RRM1.[297] Indeed, some recent evidence suggests that PARP5a/b inhibition destabilizes stress granules, as mentioned above,[34,102,118,297] and PARP5a activity is sufficient for homotypic and heterotypic droplet formation in vitro.[34] Yet other studies indicate that PARP1/2 inhibition prevents localization of IDR-containing proteins to stress granules,[31,114] which is paradoxical given that PARP1/2 are nuclear in nearly all cases. It is also unclear what would activate PARP1, although certain stresses may trigger both DNA damage and one of the four eIF2α kinases, likely HRI.[299]

One hypothesis to explain this observation is that free PAR is produced by PARP1, which translocates to the cytoplasm through an unknown mechanism (we discuss this possibility in the section Free PAR Chains, above). Although there is some evidence to support the notion of free PAR chains, endogenous PARG activity likely degrades any exposed PAR chains nearly immediately. The basal degradation of PAR chains is supported by biochemical experiments often requiring PARG inhibition to isolate and detect PAR by Western blot.[34] Another hypothesis is that nuclear stress granule proteins are PARylated by PARP1/2, exported to the cytoplasm, and incorporated into stress granules. Again, a major problem with this hypothesis is that PAR chains attached to proteins will also be targeted for degradation by PARG, which is observed in real-time dispersal of proteins to the DNA damage response machinery within minutes in cells.[17,18]

One possible explanation is that a PAR reader may shield another PARylated protein from PARG through oligomerization. For instance, a protein could be PARylated by PARP1 in the nucleus, and another protein could then bind to the attached PAR chain, preventing PARG from degrading the chain. Furthermore, if a protein is a PAR reader and a substrate for

PARylation, one could imagine that dimerization of the protein could lead to safe shuttling of the protein–PAR complex from the nucleus to the cytoplasm. However, evidence for such a mechanism is currently lacking.

Given the rapid nature of PAR synthesis and the transient nature of PAR chains in vivo, it is also plausible that poly(ADP-ribosylation) of proteins acts as a molecular trigger for stress granule formation in species that express PARPs and utilize PAR.[8] PAR readers can rapidly assemble on newly synthesized PAR chains. The incorporation of RNA could be a downstream event in PAR-mediated stress granule assembly; recent evidence suggests that RNA can supplant PAR from preformed protein–PAR droplets.[34] However, further studies are needed to determine exactly how PAR contributes to stress granule assembly.

4.6. Viral nSP3 Proteins Dissolve Stress Granules via Glycohydrolase Digestion of PAR

Stress granule dissolution is a hallmark of viral infection.[300] The initial stages of viral infection promote stress granule assembly through a viral RNA-mediated signaling cascade: protein kinase R phosphorylates eIF2α,[301–304] which stalls translation and releases mRNA from polysomes for stress granule formation (Figure 9b).[305] Cessation of translation is a survival strategy initiated by the infected cell to inhibit the production of viral proteins. Later stages of infection cause the disassembly of stress granules, presumably bypassing the translational arrest imparted by stress granule formation.[306–308] Importantly, recent studies demonstrate that stress granule dissolution is at least partially driven by PAR recognition and glycohydrolase activity embedded in viral nsP3 proteins.[36,133,137–139,309–313]

nsP3 genes are conserved, encoding multifunctional proteins that are essential for viral replication.[314] Macro domains are a shared component among nsP3 proteins, enabling robust PAR reader activity.[134] Weak PARG activity is also present within the Macro domain of some viral proteins.[137,312,315] Indeed, a recent report demonstrated that this PARG activity serves a vital role in viral infection: the PAR glycohydrolase domain of nsP3 proteins targets G3BP1 PARylation,[309] and loss of G3BP1 ADP-ribosylation leads to stress granule disassembly (Figure 9c).[102] Other studies have suggested that PARG activity within the SARS-CoV2 nsP3 protein reverses PARP9 activity,[36] indicating a potential therapeutic avenue. PARP9 has also been shown to oligomerize the E3 ubiquitin ligase DTX3L.[316] The regulation of stress granules via the catalysis of poly(ADP-ribosylation) on G3BP1 highlights the relevance of PAR in maintaining the structure of phase-separated stress granules.

4.7. PAR Chains Arrest Golgi Processing of Proteins by Sequestering PARP12 in Stress Granules

In a concomitant pathway with nsP3-mediated dissolution of stress granules, infected cells are attempting to shut down translation.[300] A recent study highlighted a separate PAR-dependent mechanism that affects PARP12,[37] a Golgi-associated MARylating PARP.[52,62] The WWE domain of PARP12 recognizes PAR produced during the viral infection;[37] this PAR reader activity drives the localization of PARP12 from the Golgi to the stress granule (Figure 9b).[37,317,318] The Golgi complex simultaneously loses its canonical ribbon morphology, and posttranslational processing of proteins is halted.[37] It is possible that

PARG activity by nsP3 proteins during viral infection reverses incorporation of PARP12 into stress granules, countermanding the cell's attempt to arrest translation. However, this hypothesis has not been tested.

4.8. Osmotic Pressure Sensing Requires Basal PAR to Maintain Liquid-Like Condensates

Yet another stress response appears to depend on PAR: osmotic pressure sensing.[319] The apoptosis-related protein ASK3 is reversibly phosphorylated when cells are exposed to osmotic stress.[320] At the same time, ASK3 condenses into liquid-like droplets.[319] Unlike other biological processes discussed, PAR is not required to form the condensates; instead, basal PAR levels appear to be required to maintain the liquid-like properties of the ASK3 condensates in vitro and in vivo.[319] Mutations of ASK3′s PBM or degradation of PAR by PARG leads to the formation of solid-like condensates that cannot be resolved through ASK3 phosphorylation,[319] indicating that the presence of PAR may help facilitate the enzymatic phosphorylation of ASK3 in condensates.

4.9. PARP5a Phase Separation May Impact Cytoskeletal Polymerization

The PARP enzymes may also undergo PS, especially PARP5a/b and their ankyrin repeats.[62] Indeed, recent evidence suggests that PARP5a undergoes PAR-independent condensation,[34] although PAR may enhance the degree of PS. In cells, PS of PARP5a/b may enable actin cytoskeletal branching by competing with Arp2/3 for binding to Arpin,[321] which antagonizes Arp2/3-mediated branching.[322,323] Indeed, PARP5a localizes to the mitotic spindle and is required for proper cytoskeletal polymerization during mitosis.[62,324–326] PARP5a is also required for the separation of telomeres during mitosis.[326,327] Given recent reports that PS occurs at telomeres, PARP5a activity may regulate the condensation at telomeres through PARylation.[328,329] A direct link between PARP5a-dependent PARylation, PS of PARP5a, and regulation of cytoskeletal activity has not yet been made.

5. ACCUMULATION OF POLY(ADP-RIBOSE) IN NEURODEGENERATIVE PATHOLOGIES

Dysregulation of PARPs or accumulation of PAR chains can have profoundly negative consequences for neurons. Hyperactive PARP1 may help cells overcome copious DNA damage sites, but this increased activity can drive error-prone repair, trigger cell death pathways, or possibly contribute to deleterious phase transitions of IDR-containing proteins. The role of PAR in cancer is well documented, and we refer the reader to recent reviews for more on this subject (refs 330, 331). Here, we will focus on how PARylation may lead to neurodegeneration by coarsening phase-separated condensates or mislocalizing IDR-containing proteins (we also refer the reader to a recent review on this subject, ref 332).

Abnormal expression of PAR metabolic enzymes is linked with a variety of rare neurological disorders. Recessive Mendelian mutations in the glycohydrolases ARH3 and TARG1 are associated with early onset neurodegeneration.[51,333] Single nucleotide polymorphisms at the MacroD2 glycohydrolase locus have also been identified in epilepsy, autism, multiple sclerosis, and schizophrenia.[334–338] Given that these mutations target PAR-degrading enzymes, it is likely that the accumulation of PAR chains is inherently

neurotoxic. This notion is further supported by a recent report linking hyperactivation of PARP1/2 to aggregation of thousands of proteins, causing the neurodegenerative disorder ataxia-telangiectasia.[339] PARylation is of course not entirely deleterious. PAR is required for proper development: PARP1/2$^{-/-}$ mice embryos die during gastrulation, and PARP5a/b$^{-/-}$ mice embryos die prior to formation of the blastocyst.[63,340] Therefore, the moderate expression level maintained by careful regulation of PARP and PARG activity is essential for healthy development and cellular homeostasis.

In neurodegeneration, the accumulation of PAR has been linked with Parkinson's disease, Amyotrophic lateral sclerosis/frontotemporal dementia (ALS/FTD), and Alzheimer's disease. Importantly, aberrant phase transitions are detected in each of these diseases: α-synuclein in Parkinson's disease,[341] RNA-binding proteins with PrLDs, including TDP-43, FUS, hnRNPA1, hnRNPA2, TAF15, EWSR1, and TIA1 in ALS/FTD,[18,120,342–346] and tau in FTD and Alzheimer's disease.[347] Dysregulation of the DNA damage response and apoptosis are also associated with neurodegeneration, and PAR is essential for these biological processes. In this section, we will review the clinical and primary research concerning the role of PAR in each of these diseases, especially in the context of the condensation of proteins.

5.1. Premature Cell Death Is Driven by PAR Accumulation in Parkinson's Disease

Parkinson's disease is driven by the pathological accumulation of misfolded α-synuclein.[348,349] The exact mechanism of Parkinson's disease progression is debated, but it is likely a confluence of α-synuclein aggregation, prion-like transmission of α-synuclein aggregates, and activation of cell death pathways.[350] Recent studies have indicated that PS of α-synuclein can seed Parkinson's-associated aggregates,[341] and it is certainly plausible that PAR chains contribute to this PS event. However, the strongest evidence for a role of PARylation in Parkinson's disease involves its contribution to the cell death pathway parthanatos.

Parthanatos is triggered by high concentrations of free PAR, which is highly cytotoxic.[112] Higher concentrations of longer PAR chains are especially damaging.[112] PAR initiates cell death through a caspase-independent mechanism.[112] Instead, free PAR induces the translocation of apoptosis inducing factor (AIF) from the mitochondria to the nucleus, where it shears DNA and triggers cell death.[113,351] Depletion of NAD^+ levels by hyperactive PARP1 also likely drive AIF recruitment to the nucleus.

In Parkinson's disease, α-synuclein aggregation and PARP1 activity promote each other, eventually driving parthanatos activation. It is thought that overactivation of PARP1 through DNA damage may initiate extensive PARylation.[352] PAR chains can then induce aggregation of α-synuclein.[352] Importantly, α-synuclein fibrils drive PARP1 activity in a devastating feedback loop, which triggers parthanatos and causes cell death of dopaminergic neurons in mice.[352,353] Moreover, transfection of human neuronal cells in culture with purified PAR chains elicits formation of toxic, cytoplasmic α-synuclein inclusions.[354] Therefore, deleterious phase transitions of α-synuclein, possibly through aberrant PAR-mediated PS, increases PARP1 activity, which eventually induces cell death through the parthanatos pathway, leading to a Parkinson's-like pathological phenotype.

Elevated PAR levels were found in the cerebrospinal fluid of Parkinson's patients,[352] indicating that this PAR-mediated mechanism is a plausible course of the disease in humans. Small-molecule inhibitors of PARP1 activity have shown promising results in cell models.[354] It may also be plausible to supplement cells with NAD^+, which appears to inhibit translocation of AIF and activation of the parthanatos cell death pathway.[355,356] Other approaches may limit PAR-mediated PS of α-synuclein, which is one of the most upstream components of the pathway.

5.2. Elevated PAR Levels Are Linked with Alzheimer's Disease

The exact cause of Alzheimer's disease is still unclear, but it is thought that amyloid-β peptides elicit tau tangles, which lead to the aggressive form of dementia observed in patients.[357] Amyloid-β fibers are required to transform tau protein into a neurotoxic state.[358,359] Tau is required for Alzheimer's pathology because amyloid-β toxicity alone is not sufficient for the dementia-like outcomes in mouse models.[360] Moreover, the toxicity of tau protein may arise from PS-mediated phase transitions.[361]

A clear mechanism between increased PAR activity and Alzheimer's disease is lacking, but evidence suggests that PAR may promote amyloid-β toxicity in a similar manner as it does with α-synuclein in Parkinson's disease. Increased PAR levels and PARP1 activity were observed in Alzheimer's patients.[362–364] Moreover, loss of PARP1 appears to ameliorate some of the canonical Alzheimer's phenotypes in mice.[365] Treatment with PARP1 inhibitors has a similarly protective effect on amyloid-β toxicity.[366–368] The inflammatory response or mitochondrial defects may be associated with PARP1 activity.[369,370] However, the exact link between how PAR chains may directly interact with tau and amyloid-β during aggregation or if the parthanatos pathway is directly involved in Alzheimer's disease remains to be tested.

5.3. PAR-Mediated Phase Separation of ALS/FTLD-Linked Proteins May Drive Disease Progression

Perhaps the clearest link between PAR-mediated PS and neurodegeneration is in ALS/FTD. Mutations in several RNA-binding proteins with PrLDs and expansions of repetitive RNA are linked with the formation of neurotoxic aggregates in patients.[371] ALS and FTD exist on a pathological spectrum; some patients display symptoms of both diseases, whereas other cases more closely align with only one of the diseases. The proteins that are linked with ALS/FTD, TDP43, FUS, hnRNPA1, and others, participate in biological processes that rely on PAR-mediated PS, especially the DNA damage response and stress granule formation.[332] Poly(ADP-ribose) chains promote in vitro PS of FUS, TDP-43, and hnRNPA1.[17,18,31,34,118,119]

PARylation may play both neuroprotective and neurotoxic roles in ALS/FTLD. For TDP-43, PAR-driven PS is initially protective, as it helps TDP-43 retain a liquid-like status in stress granules.[118] PARP5a activity is also required for FUS localization to stress granules.[34] Importantly, stress granules are distinct from the disease-associated aggregates formed by TDP-43 and FUS in ALS/FTD disease models.[118,346,372,373] Therefore, initial association with PAR may help solubilize ALS/FTD-linked proteins.

However, sustained incubation with PAR has a negative effect on ALS/FTD-linked proteins. Previous studies have noted that liquid-like granules may transition to solid- or gel-like material states through aging or percolation.[172,189,374,375] EWSR1, FUS, and TAF15 all transition to solid-like aggregates after prolonged interactions with PAR chains.[17] High concentrations of long PAR chains promote aggregation of FUS, and PAR can help disease-associated FUS mutations mature into gel-like condensates.[34] In the context of *C9ORF72*-related ALS/FTLD, PAR directly binds to arginine-rich dipeptide repeats (R-DPRs), which in turn increases their deleterious interactions with other RNA-binding proteins, including TDP-43. In fact, PAR increases poly(GR)-induced TDP-43 aggregation, contributing to the overall toxicity.[162,376] Consistent with the notion of PAR's neurotoxic effect, inhibitors of PARP1 or PARP5a activity appear to not only prevent TDP-43 aggregation and toxicity but also suppress R-DPR toxicity.[114,119,162,297,377]

As discussed above in section Stress Granules Are Nucleated by PAR Readers and PAR Chains, the source of neurotoxic PAR in ALS/FTLD is unclear. It is possible that, like in Parkinson's disease, PARP1 activity initiates the parthanatos pathway, driving cell death. Indeed, FUS and other ALS/FTLD-linked proteins are required for prompt resolution of DNA damage and cessation of PAR synthesis by PARP1.[17,266] In ALS/FTD, the parthanatos response may be driven by the sustained activity of PARP1, consuming NAD^+ and activating AIF.[263] PARP5a/b may simultaneously supply cytoplasmic PAR for oligomerization of ALS/FTD-linked proteins, suggesting that both PARP1 and PARP5a/b inhibition will be effective in ALS/FTD.[332]

It is important to note that FDA-approved PARP1 inhibitors are not ideal as therapeutics for ALS/FTD or other neurodegenerative disorders. First, FDA-approved PARP1 inhibitors are not brain-penetrant, which may limit their efficacy in ALS/FTD; and second, they are designed to kill cancer cells by trapping PARPs on DNA, which leads to cytotoxicity.[378] For applications to ALS/FTD, these properties are undesirable, and instead we seek brain-penetrant PARP1 inhibitors with minimal cytotoxicity. There is also concern that inhibiting DNA repair pathways via PARP1 inhibition in ALS/FTD patients may also be detrimental. Thus, it may be beneficial to focus on brain-penetrant PARP5a/b inhibitors for ALS/FTD, which can effectively mitigate TDP-43 neurotoxicity[297] and would not impair DNA repair pathways.

6. NEW METHODS TO STUDY THE ROLE OF PAR IN PHASE SEPARATION

An emerging theme from the literature explored in this review is that PARylation is a unique promoter and regulator of PS, especially in the context of the stress response and at DNA damage foci. In vitro experiments demonstrate that purified PAR chains directly promote PS through protein–PAR interactions,[18,34,118] and biochemical studies further show that many PS-prone proteins accept PARylation modifications.[31] In cells, PARylation activity of PARP1/2 and PARP5a/b, which synthesize the nuclear and cytoplasmic PAR chains in the cell, are required for PS at DNA damage foci and at stress granules, respectively.[17,297] The assembly of these granules appears to be temporally coordinated with PARP activation, indicating that PAR may act as a seed for PS.[18]

However, the exact role of PARylation in many of these processes is unclear. There are several major questions that the field needs to address: (1) What is the molecular mechanism of how PAR promotes PS in cells, especially which proteins are accepting PARylation modifications and which proteins are recruited by these PAR chains? (2) What exactly is the role of PARP1 in regulating cytoplasmic PAR PS processes, and is PAR a messenger to direct cytoplasmic PS in response to DNA damage stress? (3) What is the functional relevance of PAR-mediated PS, and does this PS serve a protective role in stressed conditions for the cell? (4) Are PARPs or PAR chains a therapeutic target for neurodegenerative diseases caused by pathological aggregation of PS-prone proteins?

One major challenge to study the role of PARylation in PS and neurodegeneration is the lack of commercially available tools to monitor, synthesize, and manipulate PAR chains. Unlike DNA and RNA, PAR chains of discrete lengths or with specific chemical modifications are not available for purchase from commercial sources. It is also difficult to track or target PAR in cells. These technical challenges preclude efficient and rigorous studies on PAR-mediated PS. Fortunately, recent advances in PAR technology are poised to help researchers overcome many of the obstacles that have impeded PAR-mediated PS research to date. In this section, we review exciting new PAR tools, which are summarized in Table 3.

For in vitro studies, commercially available PAR products currently consist of a mix of “long” (80–200-mer), unmodified PAR chains. However, several recent studies demonstrate that PAR chains of discrete lengths can be purified and then modified for biochemical and biophysical studies. To isolate PAR chains, the catalytic domain of PARP5a is purified and combined with NAD^+ to generate large amounts of PAR chains. After dissociating PAR from the catalytic domain with 1 M KOH, PAR chains of distinct lengths are isolated via high-performance liquid chromatography.[379] PAR chains can be further modified using copper-catalyzed alkyne–azide cycloaddition to azide-modified polymers or enzymatic labeling of the terminal ADP-ribose (ELTA) with the protein OAS1.[34,380] Because PARP5a generates linear PAR chains, these methods enable more detailed studies of cytoplasmic PAR.

One major challenge is to generate discrete versions of branched PAR, which is synthesized by PARP1.[26] A recent study demonstrated that point mutations in PARP1 alter the extent of PAR branching,[60] but this finding has not yet been leveraged to create branched PAR chains with the desired branching in a reproducible manner. Such a technology would enable biophysical and mechanistic interrogation of PARP1-mediated PS at the DNA damage site.

In PAR-mediated PS, there are proteins that accept PAR chains as modifications (hubs) and proteins that recognize PAR chains (readers) (Figure 3). Recent advances have furthered our understanding of which proteins inhabit each group. To identify PAR readers, a recent report created PAR photoaffinity probes called PARprolink by using the ELTA technology.[156] The PARprolink system enabled the robust pulldown and identification of PAR binding proteins in cells. PARprolink was added to HeLa nuclear extract in this study, but it would be more physiologically relevant to introduce the PARprolink probe into living cells. Combined with mass spectrometer studies that identify PARylated amino acids on proteins,[74,93,97,101,115,136,382,388] these two techniques can identify the PAR hubs

and readers. The main experimental challenge will be matching and mapping the hubs and readers in a robust and reproducible way, especially because there is still uncertainty about which amino acids truly accept PARylation modifications.

The difficulties in studying PAR also extend to visualization of PAR in cells. Although there are several commercially available antibodies for detection of PAR, there is wide variability in the efficacy and reproducibility of the antibodies, and they tend to recognize PAR much more efficiently than MAR. A recent report created an antibody-like protein fusion that identifies PAR chains for a variety of biochemical applications.[383] Moreover, several groups are advancing technologies to track PAR in live cells.[384–390] The recently reported PAR Tracker uses an oligomerization-dependent split nano luciferase with PAR-reading WWE domains to allow live tracking of PAR chains, which can also detect changes in PARylation levels in response to DNA damage and other stimuli.[384] Other versions of PAR-Ts can also be used to recognize certain types of PAR chains. Importantly, PAR Tracker could be used to identify whether PARylation is accumulated at cellular granules like stress granules and DNA damage foci. Clickable PAR probes have also been used to visualize PAR chains in cells.[385–387]

One important strength of the technologies to study PAR is the many enzymatic inhibitors available for PARP1, PARP5a/b, and PARG. A variety of potent small molecules have been developed that reliably inhibit these proteins, and the PARG inhibitor in particular is quite important for halting PAR degradation in cellular lysates while performing biochemical assays.[34,156] This is a unique asset in studying PAR-mediated PS, as it is difficult to inhibit other PS implicated posttranslational modifications in a similar manner.

7. CONCLUSIONS AND FUTURE DIRECTIONS

PARylation is emerging as a mechanism through which the cell can organize the response to various cellular stimuli, including DNA damage, oxidative stress, viral infection, osmotic pressure changes, and others. Synthesis of PAR chains allows the rapid assembly of IDR-containing PAR readers into phase-separated granules. By targeting certain proteins for PARylation, including PARPs and the stress granule protein G3BP1, the cell can nucleate a new granule within minutes. Importantly, the control of PAR concentration in cells through basal PARG expression allows the equally quick dissolution of granules once the stimulus has passed or resolved. Dysregulation of PAR levels is linked with a variety of neurodegenerative disorders that are thought to be caused by aberrant protein oligomerization, indicating that clinical intervention that corrects PAR levels may be effective.

Moving forward, the field should make use of new advances in PAR biology and biochemistry to further interrogate the mechanisms underlying PAR-mediated PS. Once the PAR biology toolkit is more widely available to the research community, more in-depth experiments of PAR in PS will be possible. In the context of ALS/FTD, it would be of interest to explore the effects of PAR length and PAR branching on TDP-43 PS and aggregation. While PARP inhibitors are promising potential therapeutics, there is a plethora of essential roles for PAR in cellular physiology. A better understanding of PAR-mediated

TDP-43 condensation will therefore allow us to design more specific targeted therapies to combat neuro-degeneration.

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ABBREVIATIONS

ALS	amyotrophic lateral sclerosis

DSB	double-stranded break
FET	FUS/EWSR1/TAF15
FTLD	frontotemporal lobar dementia
HR	homologous recombination
IDR	intrinsically disordered region
MAR	mono(ADP-ribose)
NAD^+	nicotinamide adenine dinucleotide
NHEJ	nonhomologous end joining
PAR	poly(ADP-ribose)
PARG	poly(ADP-ribose) glycohydrolase
PARP	poly(ADP-ribose) polymerase
PBM	poly(ADP-ribose) binding motif
PBZ	poly(ADP-ribose) binding zinc finger
PS	phase separation
RGG	arginine–glycine–glycine
RNP	ribonucleoprotein
SG	stress granule

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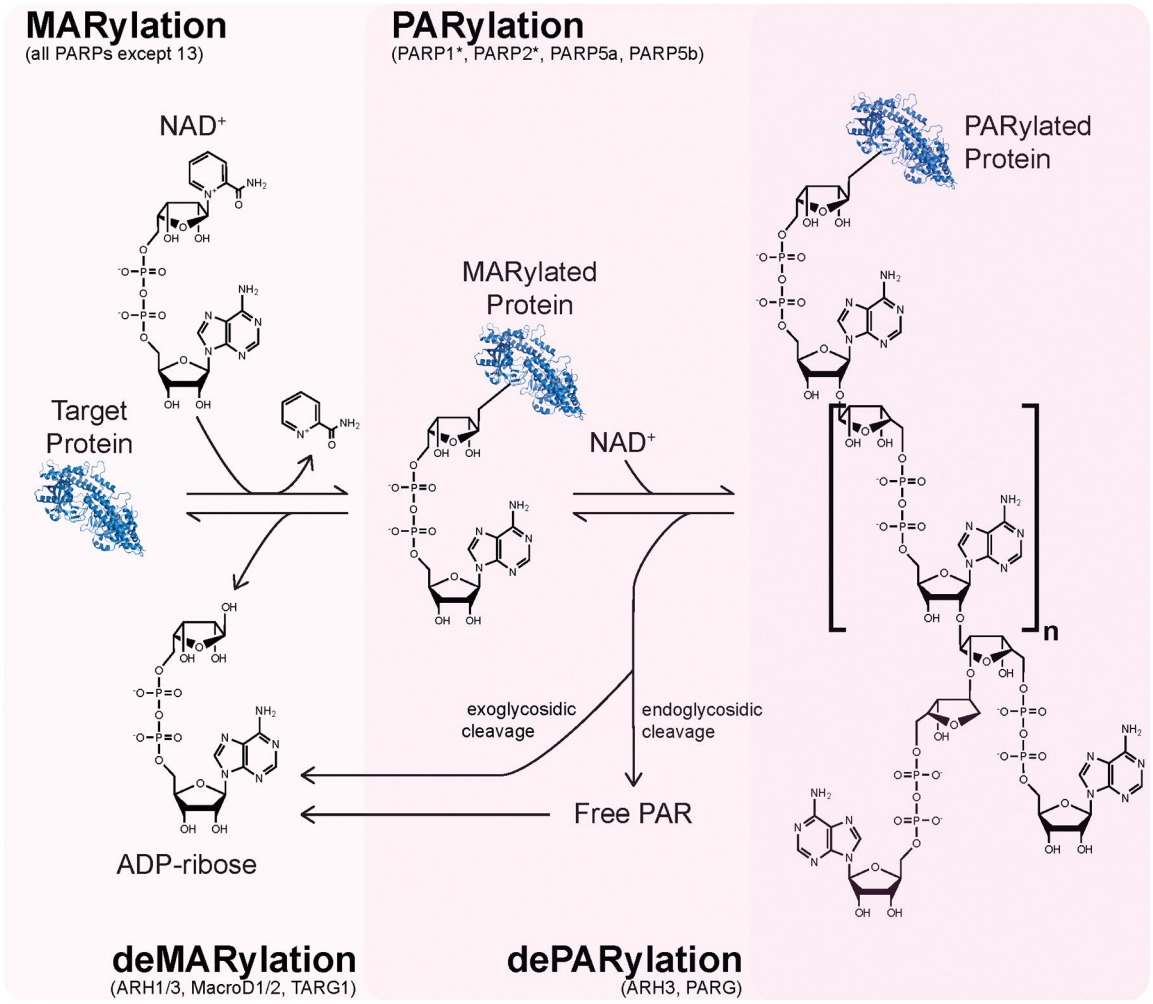

Figure 1.

The PAR cycle. Target proteins are mono- and poly-(ADP-ribosylated) (MARylated and PARylated, respectively) with nicotinamide adenine dinucleotide (NAD^+). All PARPs except PARP13 can MARylate targets, but only PARP 1/2/5a/5b can PARylate proteins. PARP1/2 are the only PARPs with reported branching activity. Proteins are dePARylated and deMARylated by PAR glycohydrolase (PARG) and PARG-like enzymes, releasing free ADP-ribose. The protein ribbon structure in this figure is the catalytic domain of PARP1 (PDB 7KK2).[39]

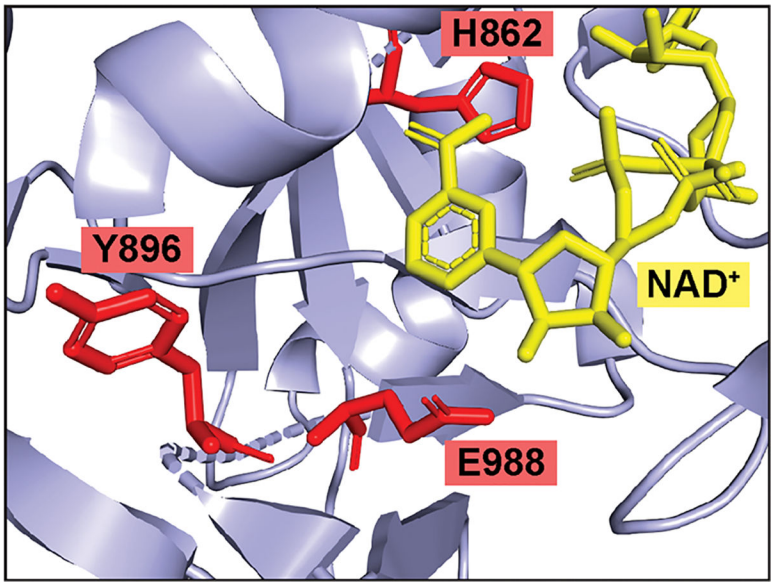

Figure 2.

H–Y–E triad of PARP1. The PARP1 ribbon structure is in light blue, H–Y–E residues are red line structures, and the NAD^+ analogue is a yellow line structure. The PDB structure is 6BHV.[245]

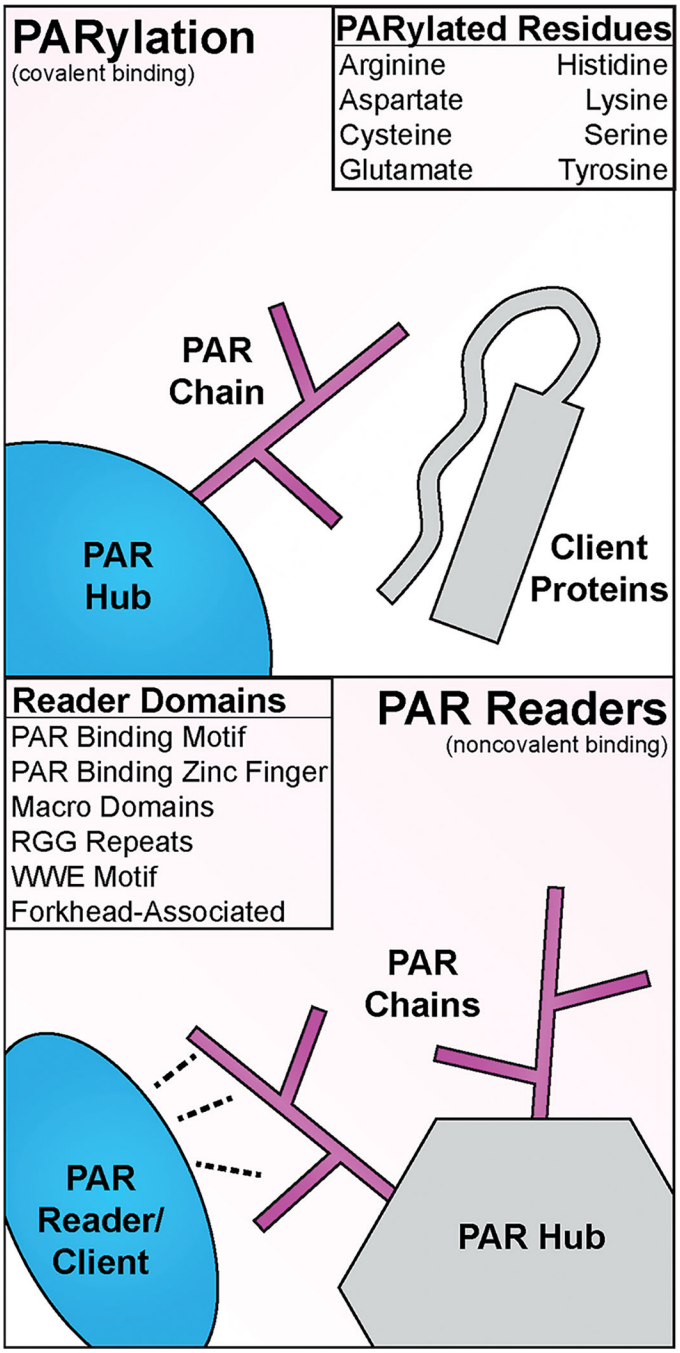

Figure 3.

Covalent and noncovalent PAR interactions. Proteins can accept PAR modifications (covalent binding, i.e., PARylation) or interact with PAR chains (noncovalent binding of PAR readers). The dashed line denotes a noncovalent PAR reader interaction.

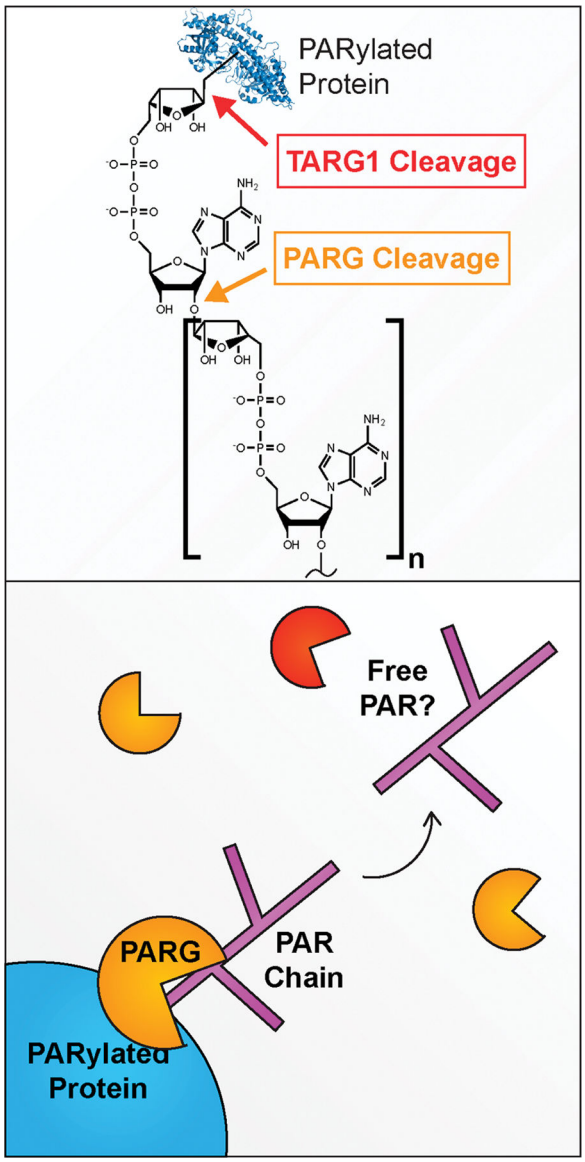

Figure 4.

A model for the production of PAR chains. In theory, PARG or TARG1 endoglycosidic cleavage of a covalently attached PAR chain may release free PAR with which other proteins may interact.

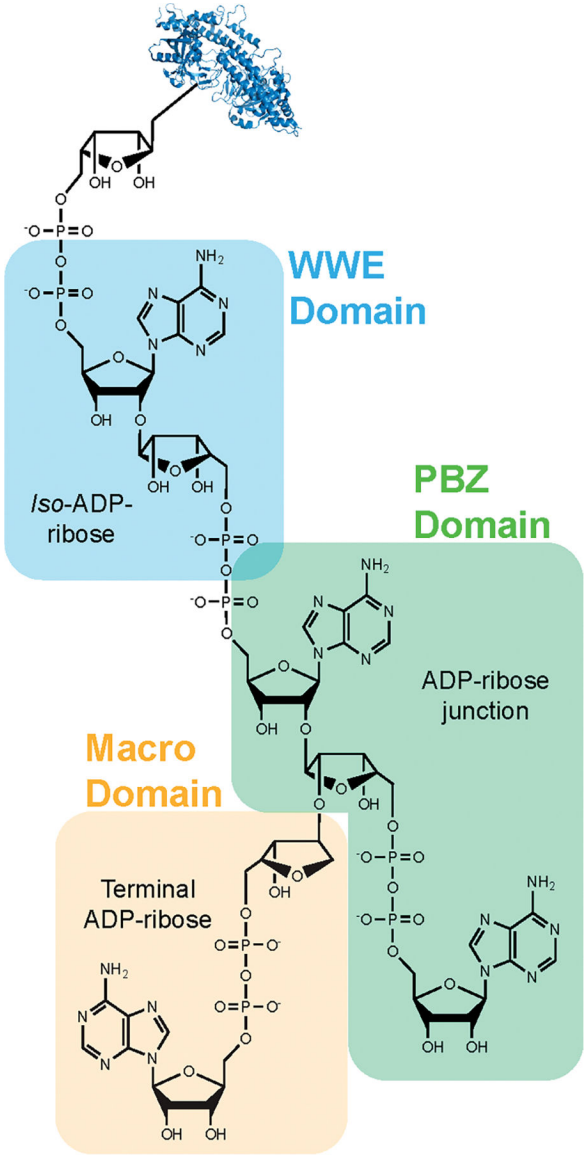

Figure 5.

PAR-reader recognition sites. The WWE domain recognizes the iso-ADP-ribose linkage, the PBZ domains recognizes a pair of ADP-riboses, and the Macro domain recognizes the terminal ADP-ribose. Other domains (e.g., PBM and RGG repeats) may recognize the negatively charged phosphate backbone.

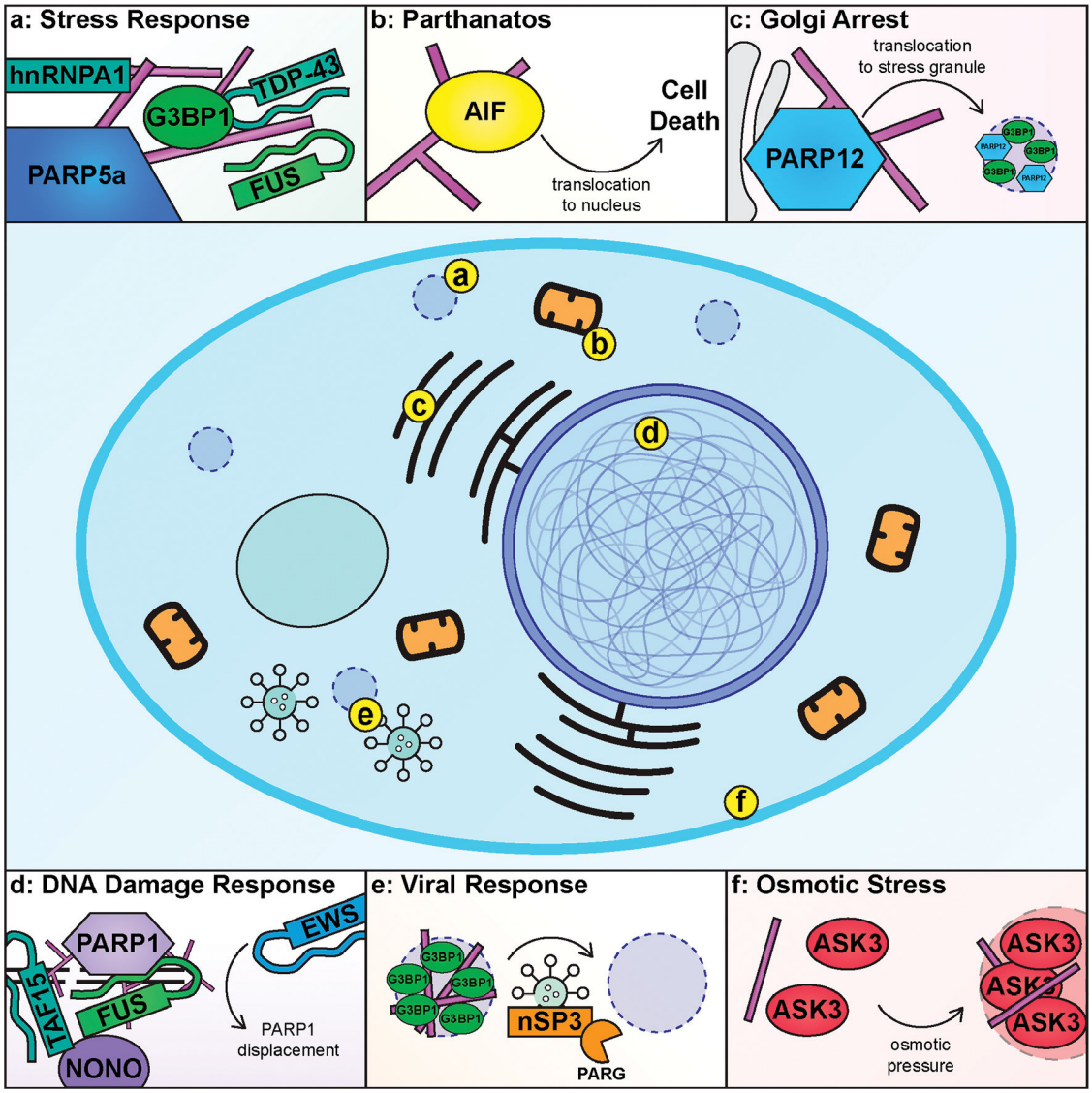

Figure 6.

The role of PAR in biological PS. (a) PARylation mediates the assembly of stress granule proteins FUS, TDP-43, G3BP1, and hnRNPA1. (b) Free PAR signals the release of AIF, which causes cell death in Parkinson's disease and other neurodegenerative pathologies. (c) PAR binding directs the translocation of Golgi-associated PARP12 to the stress granule, inhibiting Golgi function. (d) PAR chains synthesized by PARP1 initiate PS of DNA damage response proteins, including FUS, TAF15, NONO, EWSR1, and USP39. (e) PARG activity encoded in viral nsP3 proteins causes dissolution of stress granules in response to viral infection. (f) ASK3-PAR condensates respond to osmotic stress, and PAR is required for the liquid-like properties of ASK3 granules. PAR chains are pink linear or branched rods.

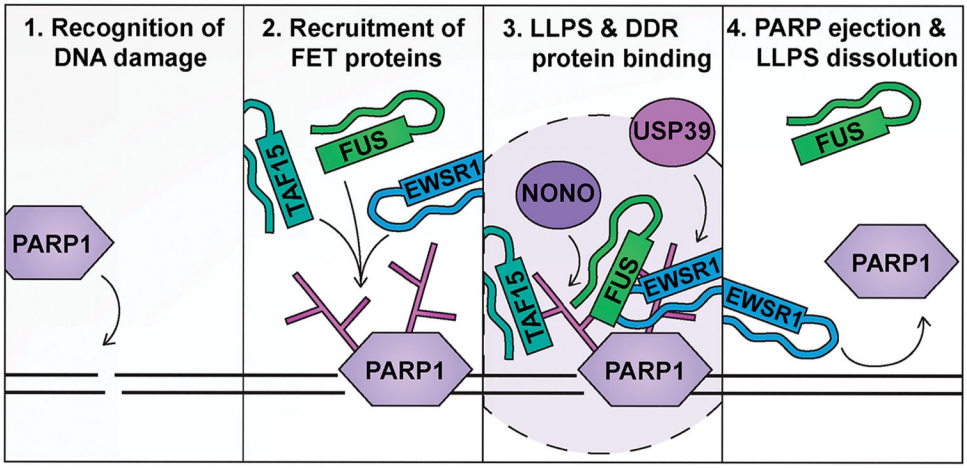

Figure 7.

The PAR-driven PS model of the DNA damage response. Recognition of double-stranded breaks by PARP1 stimulates PAR synthesis. FET family proteins (FUS, TAF15, EWSR1) and possibly USP39 are simultaneously recruited to the DNA damage site by new PAR chains, driving PS at the DNA damage site. After resolution of the DNA damage, EWSR1 and possibly FUS help eject PARP1 from the repaired DNA and dissolve the phase-separated granule. Branched pink rods are PAR chains.

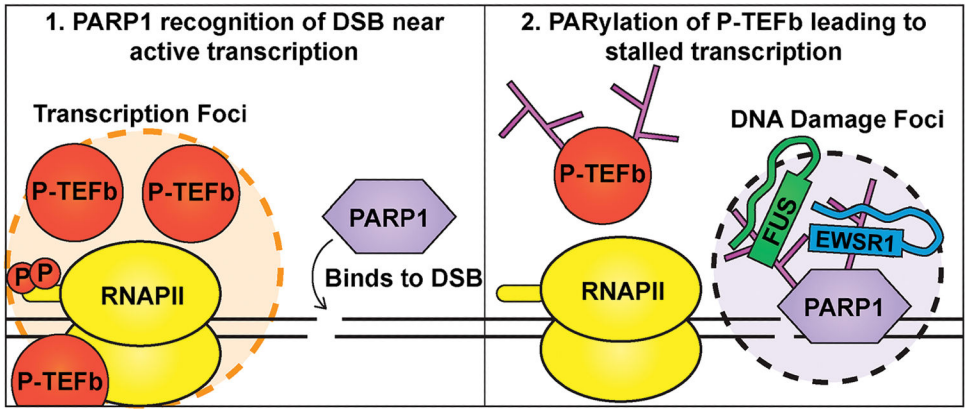

Figure 8.

Control of PS by PARylation at transcriptional DNA damage sites. If a double-stranded break is recognized near a transcriptional focus, PARP1 recruitment will antagonize transcription by PARylating P-TEFb. This action dissolves P-TEFb condensates, which stops phosphorylation of RNAP II and thus transcription. Meanwhile, a DNA damage condensate likely forms until the break is repaired. Pink rods are PAR chains.

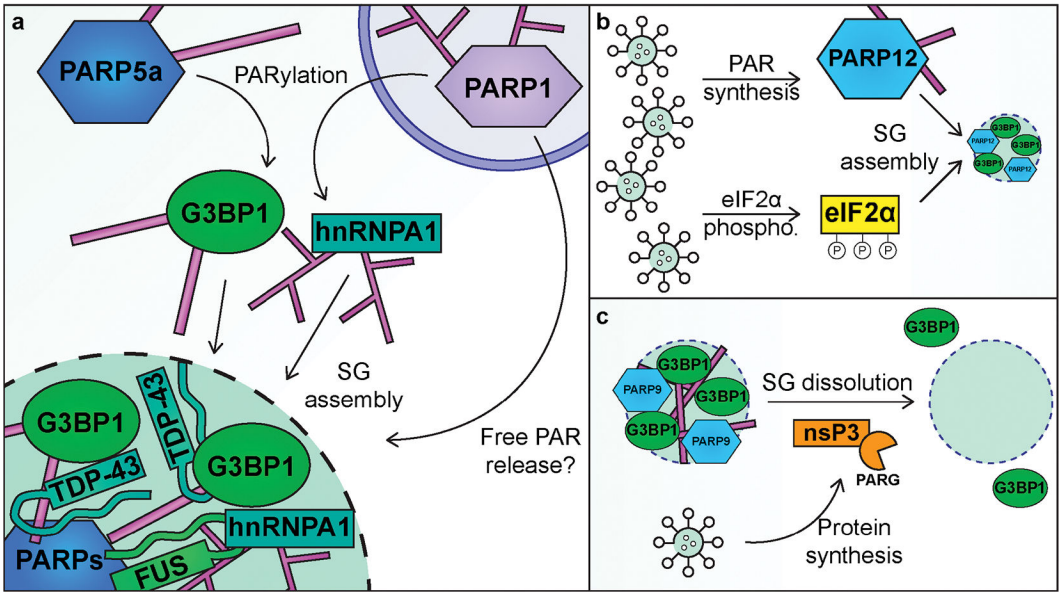

Figure 9.

PAR drives stress granule (SG) assembly. (a) PARylation of PARPs and other proteins (G3BP1 and hnRNPA1) promotes SG assembly with other proteins like FUS and TDP-43. Free PAR released from PARP1 may also contribute to SG formation. (b) Viral infection leads to simultaneous PAR production and eIF2α phosphorylation by protein kinase R, leading to Golgi arrest and SG assembly, respectively. (c) Production of viral nsP3 proteins with PARG domains degrades SGs through loss of PAR. Pink rods are PAR chains.

Table 1.

PARP Family in Humans

PARP	other names	catalytic activity	implicated in PS?[a]
PARP1	PARP, ARTD1	PARylation (+ branching)	DNA damage foci,[17] stress granules,[31] transcriptional loci[32]
PARP2	ARTD2	PARylation (+ branching)	DNA damage foci?[33]
PARP3	ARTD3	MARylation	
PARP4	vPARP, ARTD4	MARylation	
PARP5a	TNKS1, ARTD5	PARylation	stress granules[16,34]
PARP5b	TNKS2, ARTD6	PARylation	stress granules?[34]
PARP6	ARTD17	MARylation	
PARP7	tiPARP, ARTD14	MARylation	unidentified nuclear granule[35]
PARP8	ARTD16	MARylation	
PARP9	BAL1, ARTD9	MARylation	stress granules (viral response)[36]
PARP10	ARTD10	MARylation	
PARP11	ARTD11	MARylation	
PARP12	ARTD12	MARylation	stress granules[16,37]
PARP13	ZAP, ARTD13		stress granules[16,38]
PARP14	BAL2, ARTD8	MARylation	stress granules?[16]
PARP15	BAL3, ARTD7	MARylation	stress granules?[16]
PARP16	ARTD15	MARylation	

[a]PARP2 has not been directly implicated in the DNA damage foci PS, but it is required for proper PARP1 activity. PARP14 and PARP15 are both stress granule proteins, but it is unclear what, if any, function they might have in forming, regulating, or disassembling stress granules.

Table 2.

PARG Family in Humans

PARG	substrate	catalytic activity	amino acid selectivity
PARC[a]	PAR	partial	
TARG1	MAR/PAR	complete	D/E
MacroDl	MAR	complete	D/E
MacroD2	MAR	complete	D/E
ARH1	MAR	complete	R
ARH3	MAR/PAR	complete	S
ENPP1	MAR/PAR	partial	
NUDT9	PAR	partial	
NUDT16	MAR/PAR	partial	

[a]PARG has an alternatively spliced isoform that is primarily cytoplasmic.

Table 3.

New PAR Technologies to Study Phase Separation

method	description	ref
HPLC fractionation of synthesized PAR	PAR is synthesized in vitro by the catalytic domain of PARP5a, released with a strong base, and fractionated into discrete lengths by high-performance liquid chromatography	379
enzymatic labeling of the terminal ADP-ribose (ELTA)	The protein OAS1 and dATP analogues (e.g., fused to a fluorophore or affinity tag) are used to label the terminal end of the synthetic PAR chain	380
PARprolink	photoaffinity probe is attached to a synthetic PAR chain using ELTA to enable cross-linking and pulldown of PAR binding proteins in cell lysate	156
controlling PARP1 branching with active site mutations	site-directed mutagenesis of PARP1 to bias PAR chain synthesis toward short/hypobranched PAR (G972R), short/hyperbranched (Y986S), and long/hyperbranched (Y986H)	60
Click-ChIP-Seq	clickable NAD^+ analogue is used with an analogue-sensitive PARP to synthesize PAR chains that can be cross-linked, immunoprecipitated, and sequenced	279,381
mass spectrometry of PARylated peptides	there are several approaches to isolate and fragment PARylated peptides, which can lead to biases toward which residues appear to be PARylated	74,76,101,115,136,382
chimeric PAR antibodies	PAR recognizing domains are fused to the Fc domain of rabbit antibodies to better recognize PAR chains	383
PAR tracker	PAR-binding WWE domain is fused to each half of a split nano luciferase to enable live cell tracking of PAR chains	384
AO-alkyne probes	PAR-binding probe recognizes cellular PAR and contains an alkyne handle for click chemistry with a reporter	385–387