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Expanding Biological Roles of Post-translational Arginylation

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Abstract: Protein arginylation is a conserved post-translational modification in eukaryotes, involving the conjugation of arginine residues to proteins by the enzyme arginyl-tRNA transferase. Historically associated with targeted degradation, recent studies have expanded this view by uncovering its broader regulatory influence across diverse cellular functions. This review first examines the established roles of arginylation in protein degradation through the Ubiquitin-Proteasome System and Autophagy-Lysosome System. It then highlights its non-degradative functions, including the modulation of protein-protein interactions, complex assembly, protein stability, and crosstalk with other post-translational modifications. Emerging evidence supports the notion that arginylation functions in a context dependent manner, simultaneously affecting both the stability and functional behaviour of proteins. Together, these works reveal arginylation as a dynamic and versatile mechanism that extends well beyond proteolysis, positioning it as a key global regulator of cellular functioning.

Keywords: Arginylation · Biology · Degradation · Non-degradative functions · Post-translational modification

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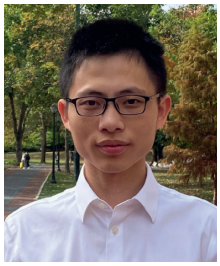

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1. Introduction

Proteins are versatile macromolecules that are responsible for coordinating a variety of cellular functions, ranging from signal regulation, metabolism, and gene expression, through to maintaining cell morphology and replication. Although a protein's primary sequence is largely built from just twenty canonical amino acids, the functional diversity of the proteome is vastly expanded through post-translational modifications (PTMs) that enable the remodelling of protein chemistries, dynamics, and interactions. Many PTMs such as protein phosphorylation, acetylation, and methylation have been well studied and have been shown to provide an additional regulatory layer that modulates protein function beyond their encoded primary sequence. Among these modifications, protein arginylation has emerged as a unique and comparatively underappreciated mechanism widely present in eukaryotic organisms.[1]

First discovered in the early 1960s,[2] arginylation involves the addition of an arginine residue to a suitable protein substrate in a ribosome-independent manner (Fig. 1). This transfer is catalysed by arginyl-tRNA transferase (ATE1), an ATP-independent enzyme that sources arginine from the cellular pool of aminoacylated Arg-tRNA.[3] Arginylation is known to occur at two different acceptor sites, namely the alpha amino group at the N-terminus

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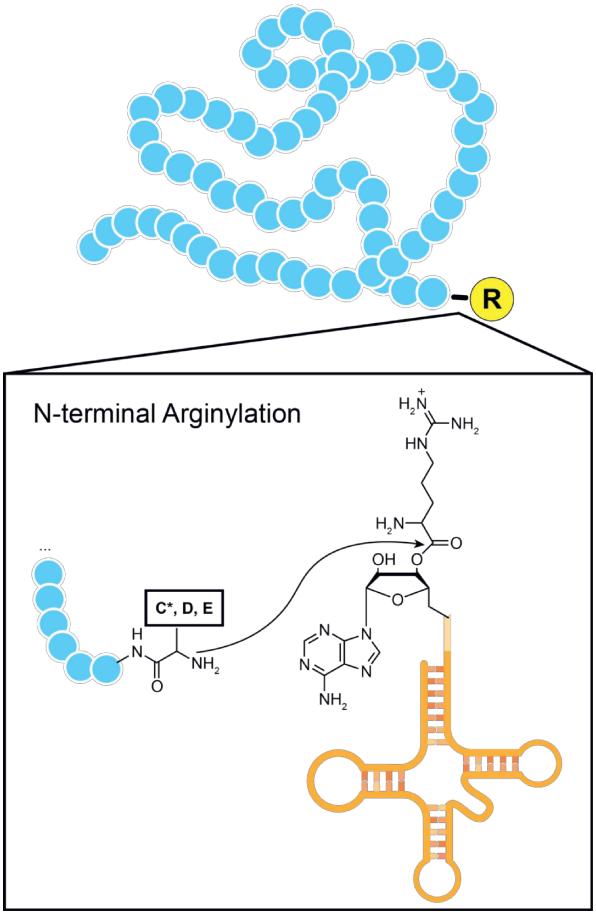

Fig. 1. Schematic representation of N-terminal protein arginylation using a simplified model of a protein substrate. The yellow circle marked 'R' represents the arginine residue added to the protein N-terminus. The inset illustrates the molecular mechanism of arginine transfer from a charged Arg-tRNA to suitable substrates bearing N-terminal oxidized cysteine (C*), aspartate (D), or glutamate (E).

of a protein or side chain carboxyl groups of internal residues.[4] Typically, these sites are defined by the presence of aspartate, glutamate, or oxidized cysteine, reflecting the preference of ATE1 for acidic residues, with recent structural studies beginning to clarify how the preferences for these substrates are established at the molecular level.[5] Additionally, these analyses have revealed that despite arginylation being a well conserved mechanism in eukaryotes, ATE1 exhibits notable differences across species that likely contribute to variations in enzyme activity. Within complex organisms, ATE1 is further diversified into multiple isoforms with different subcellular localizations as well as substrate specificities and affinities.[1d,3b,6] For instance, although ATE1 is present at lower abundance in the nucleus relative to the cytoplasm, its catalytic activity can be comparatively higher in the nuclear compartment, suggesting context dependent regulation of arginylation efficiency that may support distinct nuclear functions.[7] Adding to this complexity, the biological essentiality of ATE1 varies across species. For instance, in *Mus musculus*, ATE1 deletion results in severe developmental defects and even embryonic lethality, defining the indispensable role of arginylation in higher organisms.[8] By contrast, organisms such as *Saccharomyces cerevisiae* remain viable upon the deletion of ATE1, although with reduced environmental adaptability and disrupted stress responses.[9]

These insights reveal how protein arginylation constitutes a highly adaptable regulatory mechanism whose activity is shaped by the context of organism, substrate availability, and isoform diversity. As the field of protein arginylation has advanced, it has become increasingly clear that the functional implications of this PTM are further reaching than initially thought. However, defining true endogenous substrates and the pathways influenced by arginylation has been historically difficult, largely due to its inherently low stoichiometry and the analytical challenge in distinguishing it from the chemical equivalent of ribosomally incorporated arginine. Promisingly, however, recent technological and analytical advances have begun to overcome these barriers, revealing arginylation as a multifaceted PTM with broad impact on protein fate and ultimately cell biology.[10] Emerging evidence continues to support a model in which arginylation functions across two mechanistic arms. They are: protein degradation, classically associated with N-degron degradation pathways, and a rapidly expanding suite of non-degradative functions that modulate protein localization, assembly, signalling, or stabilization. Importantly, these functional categories are not mutually exclusive, and increasing evidence suggests that degradative and non-degradative roles of arginylation can intersect in their functions. This framework provides a useful lens through which to examine the diverse and increasingly appreciated biological roles of this PTM and will be further explored in this review.

2. Degradation Pathways of Arginylated Proteins

Degradation pathways that regulate protein half-life *in vivo* are central to protein quality control[11] and the maintenance of cellular homeostasis.[12] These systems are responsible for the removal of misfolded, damaged, or aggregated proteins that could otherwise accumulate and disrupt cellular function. In eukaryotic cells, this quality control is primarily achieved through two major proteolytic systems: the ubiquitin-proteasome system (UPS) and the autophagy-lysosome system (ALS).[13] Both systems rely on the presence of degradation signals, known as degrons, which are recognized by dedicated recognin proteins that facilitate the targeted elimination of substrates. One prominent class of degrons is the N-degron, formerly referred to as the N-end rule, which was first reported in 1986 and derives its name from the fact that the residue at the N-terminus of the protein can act as a degradation signal.[14] Operating within this degradation framework, the Arg/N-degron pathway serves as a specialized mechanism that identifies proteins bearing N-terminal arginine. Protein arginylation acts as a key post-translational tagging mechanism in this pathway, whereby the addition of an N-terminal arginine can flag substrates for degradation. This PTM therefore enables their selective routing to either the UPS or ALS, highlighting the versatile and context-dependent role of arginylation in proteolytic control (Fig. 2).

2.1 *The Arg/N-degron Pathway*

The Arg/N-degron pathway, in which arginylation functions as a key degradation signal, represents one of the major branches of the N-degron pathways. In this pathway, co-translational removal of the N-terminal methionine by Met-aminopeptidases exposes a downstream destabilizing residue, whose fate is then regulated in a hierarchical manner.[15] Tertiary destabilizing residues must first be converted into secondary destabilizing residues before they become suitable substrates for arginylation. More specifically, Asn and Gln are converted to Asp and Glu respectively by deamidases, whereas Cys (C) can undergo oxidation *via* cysteamine (2-aminoethanethiol) dioxygenase to form oxidized Cys (C*).[16] These secondary residues are then directly recognized and Nt-arginylated by ATE1, which conjugates the primary destabilizing residue arginine, allowing it to be recognized by an N-recognin.[17]

The N-recognins of the Arg/N-degron pathway within the UPS comprise a set of E3 ubiquitin ligases, including Ubr1, Ubr2, Ubr4, and Ubr5 in mammals, all of which contain a ~70-amino-acid zinc-finger–like UBR box that mediates Arg recognition, as well as the Prt1 and Prt6 E3 ligases in plants.[12,15,18] This set of

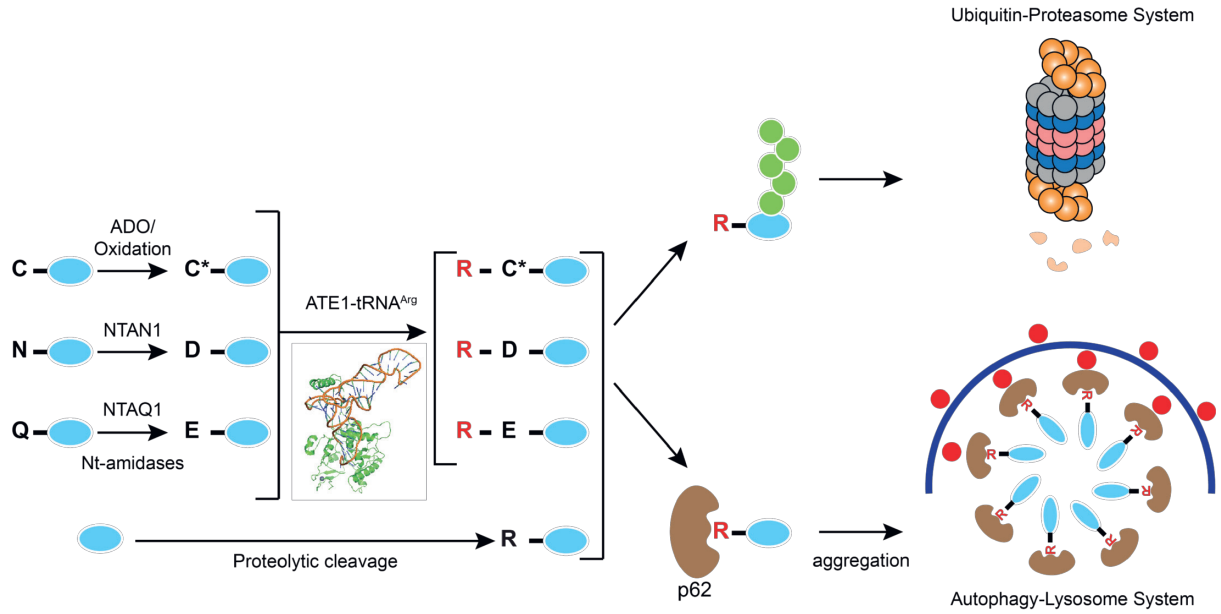

Fig. 2. Illustration of degradation pathways for proteins (blue ovals) marked through Nt-arginylation. Tertiary destabilizing residues, cysteine (C), asparagine (N), and glutamine (Q), undergo conversion to secondary destabilizing residues oxidized cysteine (C*), aspartate (D), and glutamate (E). Proteins with N-terminal secondary residues are recognized by ATE1 which catalyzes the transfer of an arginine residue (R) from Arg-tRNA to the targeted protein to produce an N-terminal primary destabilizing residue. N-terminal arginine residues can also be revealed by proteolytical cleavage to generate neo-N-termini. Arginylated proteins are then routed to one of two primary degradation pathways. In the Ubiquitin-Proteasome system (top right), arginylated proteins are polyubiquitinated (green circles) and degraded by the 26S proteasome. Alternatively, arginylated proteins are recognized by the autophagy adaptor p62/SQSTM1, which promotes aggregate formation and delivery to autophagosomes *via* LC3 (red circles) interactions leading to degradation *via* the autophagy-lysosome system (bottom right).

E3 ubiquitin ligases facilitates the covalent conjugation of ubiquitin to recognized protein substrates and facilitates their processive degradation by the 26S proteasome.[15] Several proteins are specifically routed through this pathway because of ATE1-mediated arginylation. Example substrates known to undergo targeted proteasomal degradation *via* this mechanism include members of the regulator of G-protein signalling (RGS) family, such as RGS4, RGS5, and RGS16, as well as cytokine IL-32, all of which harbour an N-terminal Cys that undergoes oxidation and arginylation prior to recognition by E3 ubiquitin ligases.[16c,16d,19] Earlier works exploring this process quantitatively assessed degradation through pulse-chase experiments in arginylation proficient and deficient cell lines.[16d] In this study, transient expression of RGS5 revealed a marked difference in protein stability, with only ~0.2% of the protein detectable after 60 minutes in ATE1$^{+/+}$ cells, compared to 71% in ATE1$^{-/-}$ strains. Similarly, rapid degradation was observed for RGS4, where degradation was substantially impaired in the absence of ATE1. These observations suggest that ATE1 mediated arginylation can drive highly efficient proteasomal degradation for specific substrates. In line with this, additional arginylated proteins have been implicated in targeted degradation *via* the UPS system, including proapoptotic protein fragments[20] and neurodegeneration-associated protein fragments such as α-synuclein with C-terminal fragments bearing Nt-Gln, trans-activation response element DNA-binding protein 43 (TDP43) and amyloid plaque bearing Nt-Asp, as well as Tau generated C-terminal fragments bearing Nt-Glu.[21]

However, while the identity of the N-terminal residue and subsequent arginylation play a central role in determining substrate fate *via* this pathway, it is increasingly evident that this alone does not dictate degradation outcomes. Factors such as translation and protein folding dynamics, accessibility of a ubiquitin accepting lysine, local structure, and nucleotide sequence can significantly influence degradation kinetics. For instance, distinct arginylated β- and γ- actin isoforms, despite being nearly identical in their amino acid sequence, exhibit significantly different degradation rates due to differences in their mRNA sequences and secondary structure that affect translation kinetics.[22] Slower translation of certain isoforms can lead to increased exposure of normally buried K18 residues, rendering them susceptible to ubiquitination by E3 ligases and subsequent proteasomal degradation. These findings suggest that the interplay between translation dynamics, protein folding, and degron exposure adds an additional layer to the complexity of degradation pathways and can work in concert with arginylation to fine-tune substrate fate.

2.2 *Degradation Through the Autophagy-lysosome System*

While the UPS pathway constitutes a primary method for the degradation of proteins earmarked by arginylation, it is not the sole degradative pathway. Arginylated substrates can also be degraded in parallel through the ALS where the selective autophagy adaptor p62/SQSTM1 can act as a non-E3 Arg/N-recognin by directly binding N-terminal arginine and shuttling the tagged protein toward lysosomal degradation.[23]

This alternative branch was described by Cha-Molstad *et al.*[23a] in 2015 using the endoplasmic reticulum (ER) chaperone BiP as a model. When under stress conditions, such as proteasome inhibition or the presence of foreign double-stranded DNA in the cytosol, BiP is retrotranslocated to the cytosol and Nt-arginylated by ATE1. The resulting arginylated BiP (R-BiP) is then recognized and binds to the ZZ domain of p62, triggering allosterically mediated oligomerization and aggregate formation. These p62-R-BiP aggregates are then targeted to LC3 on nascent autophagosomes, ultimately leading to the targeted lysosomal mediated degradation of p62-R-BiP. Subsequent work showed that N-terminal arginine functions as a bimodal degron, capable of directing arginylated proteins either to the UPS or ALS, depending on the physiological state of the cell.[24] Effectively, the bound p62/SQSTM1 serves as an autophagic N-recognin, providing an alternative method of clearance for ar-

ginylated proteins when UPS mediated degradation is impaired or overwhelmed.[23a,24,25]

3. Non-degradative Biological Roles

Although protein arginylation was initially regarded as a degradation signal within the N-degron pathway, an expanding body of work has begun to fundamentally reshape this view. Recent studies in the field are beginning to reveal that ATE1-mediated arginylation can also modulate protein-protein interactions, complex assembly, protein localization, and PTM crosstalk in ways that do not invoke proteolytic turnover, revealing arginylation as a global regulator of proteome activity rather than solely a degradation pathway intermediate. However, it is worth noting that arginylation often exerts its influence simultaneously, altering both protein function and half-life to enable context specific fine-tuning of cellular responses. This emerging paradigm shift highlights a far broader functional landscape for arginylation and brings to light the need to explore its diverse non-degradative activities across various biological pathways.

3.1 *Regulation of Protein-Protein Interactions, Stability, and Complex Assembly*

Building on this broadened perspective, one class of non-degradative arginylation lies in its capacity to modulate stability, protein-protein interactions, and the assembly of protein complexes. The introduction of arginine residues at defined positions can reshape a protein's interaction interface, influence partner binding, complex stability, and structural conformation.

Arginylation of β-actin at its N-terminus (Asp3) represents a foundational example of how this PTM can modulate protein-protein interactions and influence complex formation without mounting degradation pathways. Previous work has found that modification at this site significantly alters actin filament dynamics by reducing both polymerization rates, as well as impairing Arp2/3 mediated branch nucleation, resulting in more sparsely organized actin networks.[26] Beyond polymerization, arginylation of β-actin also modulates the interaction between actin and myosin II by weakening its binding affinity to the basic head domain of myosin.[27] As a result, the reduced attachment time of arginylated filaments leads to diminished contractile force and recruitment, facilitating the fine-tuning of cytoskeletal architecture, force generation, and cell motility.

Along with actin, protein arginylation also modulates the microtubule cytoskeleton through the direct regulation of tubulin dynamics. A recent study has identified arginylation of α-tubulin and β-tubulin to occur at Glu77 and Asp74 respectively, surface exposed residues critical for protein-protein interactions.[28] In arginylation deficient ATE1$^{-/-}$ cells, it was observed that microtubule growth rates were slower and stability increased. These changes correlated with the observed enhanced binding of microtubule associated protein 1S (MAP1S), a microtubule stabilizing protein, suggesting that arginylation at Glu77 acts as a regulatory switch that limits MAP1S association.

ATE1-mediated arginylation has also been implicated in regulating the stability and aggregation behaviour of proteins associated with Alzheimer's disease (AD) and other neurodegenerative diseases.[29] Since 2013, it has been reported that several aggregation prone proteins and fragments such as tau (Glu3-Tau-2N^{124}), α-synuclein (Gln79), β-amyloid (Asp1 at N-terminal), and TDP43 (Arg208, Asp219, and Asp247) are selectively degraded by the Arg/N-degron pathway (ATE1-dependent) and result in short-lived proteins.[29] Later studies from Kashina and coworkers expanded the scope of arginylation and showed that arginylated α-synuclein (Asp46 and Asp83)[30] also prevents α-syn aggregation and suggests that this PTM can prevent pathological aggregation and neurotoxicity.[30b,31] Notably, brain specific ATE1 knockouts in mice exacerbated neurodegenerative symptoms relative to the wild-type, highlighting the physiological relevance of this pathway in neuronal health.[30b] Together, these results indicate that ATE1 and the Arg/N-degron pathway are likely to play a beneficial role in neurodegenerative disorders, like AD,[29b] and demonstrate the interplay between the degradative role of arginylation and broader physiological outcomes.

Arginylation has also been shown to affect protein behaviour by promoting protein dimerization and dynamic localization, as seen by the ER chaperone, calreticulin (CALR).[32] Under cellular stress conditions, proteolytically processed CALR has been observed to retrotranslocate from the ER to the cytosol, where it undergoes arginylation at its neo N-terminus (E18) and forms homodimers, an interaction not seen in the non-arginylated form.[32a] These dimers are now functionally distinct from the non-arginylated counterpart, as they are recruited into stress granules where they act as a scaffold for ribonucleoprotein complex assembly.[32a] In the absence of arginylation, CALR fails to properly localize to stress granules, resulting in impaired granule formation and reduced cell viability under stress conditions.[32a] However, in conditions where the stressor exceeds the cell survival threshold, arginylated CALR appears to associate with the cell membrane and operate as a pre-apoptotic signal.[33] In this context, ATE1$^{-/-}$ strains exhibited reduced susceptibility to apoptosis, pointing out that arginylation could potentially operate as a molecular switch that balances stress adaptations with programmed cell death. Interestingly, in addition to altering the localization and interaction potential of CALR, arginylation was also found to increase its half-life, a striking deviation from the conventional expectation for N-terminal arginylation signals for degradation.[34] Together, these studies of CALR have demonstrated how a single target, can reveal the complicated nature of arginylation and highlights its versatility as a context dependent regulatory mechanism.

3.2 *Involvement of Arginylation in PTM Crosstalk and Combinatorial Regulation*

Protein arginylation is increasingly becoming recognized as part of a broader interconnected network of PTMs that work together to reshape protein behaviour and cellular outcomes. Rather than operating in isolation, arginylation often intersects with other PTMs either sequentially, cooperatively, or even competitively to fine tune protein function.

As mentioned above in the context of N-end rule mediated degradation (See section 2), proteolytic processing plays a pivotal role in generating neo-N-termini suitable for subsequent arginylation. However, while this exposure can generate degradative signals, it does not invariably commit these substrates to proteosomal elimination. Instead, it can reveal internal arginylation sites that can operate within the non-degradative arm of the arginylation framework. A prime example of this is the talin protein, which was initially found to be arginylated at Ala1903 *in vivo*.[35] Subsequent work revealed that this modification occurs after calpain-mediated cleavage at that site, generating the 70 kDa C-terminal fragment (VAD) and plays a role in establishing and maintaining cell-cell adhesion.[36] However, it was also found that arginylation of the VAD fragment also modestly reduced its half-life, demonstrating the interwoven nature of the degradative and non-degradative roles of this PTM.

Arginylation also regulates protein fate through mutually exclusive crosstalk with other PTMs, often by targeting the same structural motif. In these instances, it functions as part of a reciprocal modification pair, where the addition of one PTM precludes the addition of the other, enabling context-dependent control of protein activity. A key example of this has been observed on the N-terminus of actin (Asp2 on β-actin), where its modification is known to play a crucial role in cytoskeletal organization and cell migration.[26a,37] Recent work by Chin *et al*.[26b]

compared N-terminally arginylated, acetylated, and unprocessed forms of β-actin and revealed that each of these mutually exclusive N-terminal states conferred unique polymerization properties and interactions with actin-binding proteins. This dynamic relationship with acetylation highlights how these PTMs can act synergistically, allowing cells to selectively tune actin in response to changing physiological needs.

As mentioned earlier in this review, N-terminal cysteines on substrates, such as the suite of RGS proteins, can undergo oxidation and become suitable substrates for ATE1. Beyond its role in N-degron mediated degradation, this oxidation itself represents a PTM that potentially functions as a sensor for oxidative or nitrosative stress.[19a] In the context of the RGS proteins, arginylation acts as an interpreter of redox state, reading oxidation modifications and translating them into downstream biological outcomes. This case reveals a broader model where oxidation and arginylation function as a coordinated PTM pair and further elucidates the participation of arginylation in a combinatorial PTM network.

The biological significance of arginylation may extend beyond the initial addition of the residue itself, as these arginine residues (translational and post-translational) are susceptible to modifications such as mono- and dimethylation.[38] An earlier study had demonstrated the potential for ATE1-mediated arginylation to serve as a precursor to such downstream modifications, identifying dimethylated arginylation on the I87 residue of α-actin and on a shared peptide of heat shock proteins 90 and 84.[7] While the functional consequences of these methylation events require further investigation, they raise the exciting possibility that arginylation may serve as a landing pad for additional PTMs, adding another new regulatory layer to PTM crosstalk.

3.3 *Emerging Functions*

While considerable progress has been made in characterizing both degradative and non-degrative roles of protein arginylation (Table 1), several functions that are less clearly defined are emerging. Previous work is beginning to extend into unexpected territories such as energy metabolism, cell death, chromatin organization, and even disease pathology. Although these areas are less understood, they highlight the involvement of this PTM in yet more fundamental cellular pathways.

One novel subfield of arginylation was uncovered by Jiang *et al.*,[39] who identified that a small proportion of ATE1 enzyme localizes to the mitochondria, suggesting a regulatory role of mitochondrial proteins. Later, it was shown that oxidative stress promotes this translocation of ATE1, where it is essential in triggering cell death through the mitochondria dependent apoptotic pathway.[40] In addition to this stress response, arginylation of the mitochondrial protein, SSBP, was also found to occur on the Glu17 residue in human cells.[41] When compared with unmodified SSBP, its arginylation greatly increased mitochondrial bioenergetics in aspects of maximal respiration and spare capacity.

As previously discussed with the modification of actin and tubulin proteins, arginylation can have a significant impact on cell architectures, and earlier work has now unveiled that this influence could also be present in the nucleus.[7] Several proteins known to affect nuclear structure, such as β actin (D51), histone H2A.1 (S41), histone H2B (A59), histone H4 (T136 and V116), and vimentin (V224), have been shown to be suitable substrates of protein arginylation *in vivo*. Supporting the involvement of arginylation in maintaining nuclear morphology, microscopy imaging of ATE1 knockout mouse embryonic fibroblasts revealed significantly reduced nuclear size compared to its wild-type counterpart, thought to result from changes in chromatin organization.[7]

Moreover, the functional influence of arginylation may extend even into clinically relevant contexts. Recent studies have implicated ATE1 and arginylation machinery in viral infections as has been seen with HIV-1[42] and SARS-CoV-2,[43] suggesting that it could be possible that the pathway is co-opted to regulate host-pathogen interactions. Additionally, protein arginylation has been observed in the parasite, *Trypanosoma cruzi*, the causative agent of Chagas disease,[44] where it may possibly contribute to parasite survival or virulence. These examples hint at a previously under-recognized function of protein arginylation biology and may hold promise as a novel entry point for therapeutic intervention of these diseases, underscoring the importance of further investigating arginylation in disease contexts.

4. Conclusions and Future Perspectives

Over the past couple of decades, protein arginylation has evolved beyond its early designation as an intermediate within the N-degron pathway to a complex and multifaceted post-translational mechanism influencing a wide variety of cellular processes. As highlighted in this review, arginylation is now recognized to regulate many different non-degradative roles, including protein oligomerization, subcellular localization, stability, and interplay with other PTMs. Importantly, the examples covered here only represent a fraction of the biological impact of arginylation, with many roles, substrates, and regulatory mechanisms awaiting discovery. Despite these considerable advances, our understanding of the full biological scope of arginylation remains limited. However, global profiling strategies that combine mass spectrometry with developed pan-arginylation antibodies and isotopic labelling, large-scale genetic interaction screens, and machine learning have begun to fill this gap by uncovering hundreds of putative arginylation targets across diverse biological pathways.[4b,41,45] These datasets not only point to the widespread potential for the regulatory influence of protein arginylation but also provide a valuable resource for hypothesis generation and substrate validation. It is noteworthy that current profiling efforts [41,45b] mostly focus on the arginylation detection/discovery but not arginylation quantification due to low abundance. Therefore, the arginylation stoichiometry is a quite understudied area. Some studies tried to quantify arginylation levels using overexpressed proteins: overexpressed CALR was determined to have 1.7–2.5% arginylation in 3 conditions,[32c] SSBP showed only one MS2 spectrum of the arginylated E17 peptide among all detected E17 peptides.[41] In addition, endogenous arginylation of α-Syn in patients' brains was relatively quantified compared to GAPDH levels using anti-arginylation antibodies.[31]

Importantly, the expanding functional landscape of protein arginylation opens promising avenues for therapeutic exploration. Emerging findings have implicated ATE1-mediated arginylation in critical processes such as mitochondrial regulation, apoptosis, chromatin organization, embryonic development, and neurodegeneration. These connections suggest that modulating protein arginylation or targeting specific substrates could offer novel treatment strategies for diseases marked by proteostatic imbalance or disrupted protein signalling. Furthermore, future research aimed at identifying distinct arginylation patterns may uncover valuable diagnostic or prognostic biomarkers, underscoring the clinical potential of this versatile PTM. Since arginylation is canonically involved in the Arg/N-degron pathway, it may hold huge therapeutic potential for targeted protein degradation.

While the traditional dichotomy of degradative versus non-degradative roles has provided a valuable framework for organizing our understanding of arginylation, the growing diversity of its functions suggests that this classification may represent only part of a far more intricate regulatory map. As the field progresses, there has been a clear shift toward a more holistic perspective that embraces the context-dependent and interconnected nature of arginylation within cellular networks.

Table 1. Representative examples of arginylated proteins, their known arginylation sites, associated biological functions, and corresponding references.

Protein/substrate	Arginylation site	Biological function	Mechanism Type	Reference
RGS4, RGS5, RGS16	Nt-Cysteine(Cys-2)	Marking for proteolysis	UPS	16d
Proapoptotic protein fragments	Asp-BRCA1, Cys-RIPK1, Cys-TRAF1, Asp-BCLXL, and Asp-EPHA4	Marking for proteolysis	UPS	20
BiP	Glu19	Marking for proteolysis	ALS	23a
β-actin	Nt-Asp(Asp3)	Altered actin filament dynamics and weaker binding to myosin	Non-degrative role	26a, 26b, 27
α-tubulin and β-tubulin	Glu77(α), Asp74(β)	Limiting the binding of MAP1S to microtubules	Non-degradative role	28
α-synuclein	Gln79, Asp46 and Asp83	Marking for proteolysis, prevent pathological aggregation and neurotoxicity	Both non-degradative role and degradative role	29b, 30b, 31
TDP43	Arg208, Asp219, and Asp247	UPS for TDP43 and autophagy for insoluble aggregates	UPS and ALS	29b
β-amyloid	Nt-Asp(Asp1)	Marking for proteolysis	UPS	29b
CALR	Glu18	Forming dimers with new function as scaffold for ribonucleoprotein complex assembly	Non-degrative role	32a
talin protein	Ala1903	Reducing protein half-life, assist in establishing and maintaining cell-cell adhesion	Both non-degradative role and degradative role	35, 36
α-actin	Ile87	Precursor to such downstream modifications	Non-degradative role	7
SSBP	Glu17	Increased mitochondrial bioenergetics in aspects of maximal respiration and spare capacity	Non-degradative role	41

Further adopting this view will be essential for fully capturing the complexity and versatility of this robust and multifaceted PTM.

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Author Contributions

Conceptualization, D. S, C. F, B. A. G, and Z. L; Writing – Original Draft, D. S, C. F, and Z. L; Writing – Review and Editing, D. S, C. F, B. A. G, and Z. L. All authors have read and agreed to the published version of the manuscript.

Abbreviation	Definition
PTM	Post-translational modification
ATE1	Arginyl-tRNA transferase
UPS	Ubiquitin-proteasome system
ALS	Autophagy-lysosome system
RGS	Regulators of G-protein signaling
ER	Endoplasmic reticulum
TDP43	Trans-activation response element DNA-binding protein 43
MAP1S	Microtubule associated protein 1S
AD	Alzheimer's Disease
CALR	Calreticulin
VAD	70kDa C-terminal fragment of talin protein

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