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Research Article

Small Proteins: The Missing Link in Cellular Stress Response

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Abstract

Cells constantly encounter diverse stressors, including heat, oxidative damage, nutrient deprivation, and infection. Classical models of stress adaptation have emphasized canonical proteins of 100 or more amino acids, overlooking a vast repertoire of small proteins encoded by short open reading frames (sORFs). Recent advances in ribosome profiling and specialized proteomics have revealed that smORF-derived peptides, often fewer than 100 codons, are widespread across bacteria, archaea, and eukaryotes. Far from being translational "noise," small proteins are structurally diverse, biochemically stable, and functionally integrated into stress response networks. They act as rapid regulators of membrane integrity, proteostasis, and signaling, frequently serving as interaction modules that modulate larger complexes or rewire pathways with minimal structural footprint. Their evolutionary landscape is dual: deeply conserved subsets sustain essential stress functions, while lineage-specific microproteins emerge de novo to fine-tune adaptation in particular ecological contexts. This dynamic balance of conservation and innovation positions small proteins as critical yet underappreciated components of cellular resilience. By enabling swift, reversible proteomic remodeling, they provide a missing link between transcriptional control and immediate stress adaptation. Recognition of the "small proteome" challenges long-standing paradigms and opens new avenues for understanding and manipulating stress biology across all domains of life.

Keywords: *Small proteins, smORFs / microproteins, Cellular stress response, Ribosome profiling (Ribo-seq), Proteostasis regulation.*

1.0 Introduction

Cells are continually exposed to environmental and endogenous stressors, including heat, oxidative damage, nutrient deprivation, misfolded proteins, and infection. For decades, the molecular logic of these responses was framed almost exclusively in terms of canonical proteins larger than 100 amino acids and well-defined signaling pathways.

This view is now seen as limited. Across all domains of life—bacteria, archaea, and eukaryotes—genomes contain a massive, often ignored layer of small proteins and smORF-encoded peptides. These molecules function as versatile regulators for membrane stability, proteostasis, and stress responses (Jordan et al., 2023); (Y. Li et al., 2021); (Schlesinger & Elsässer, 2021); (Miravet-Verde et al., 2019). While only a few have been studied in detail, they are widespread and structurally diverse.

Generally, small proteins are defined as products of short open reading frames (sORFs) under 100 codons, though bacterial studies often use a 50-amino-acid limit due to detection challenges (Jordan et al., 2023); (Ahrens et al., 2021); (Weaver et al., 2019); (Miravet-Verde et al., 2019). Their names vary based on their origin: "smORFs" or "sORFs" describe the genomic segments, while the resulting products are called SEPs, microproteins, or sPEPs (Schlesinger & Elsässer, 2021); (Leong et al., 2022); (Valdivia-Francia & Sendoel, 2024); (Miravet-Verde et al., 2019). Additionally, uORFs in 5′ leader sequences can produce peptides while simultaneously controlling the translation of downstream genes. These sequences are found in mRNAs, noncoding RNAs, and even within other genes, significantly outnumbering

traditional protein-coding regions (Y. Li et al., 2021); (Schlesinger & Elsässer, 2021); (Chothani et al., 2023); (Miravet-Verde et al., 2019).

For much of the genomic era, this entire class of proteins remained largely invisible. Automated annotation pipelines imposed minimum ORF length thresholds of approximately100 amino acids, to avoid spurious predictions, effectively excluding most smORFs (Chothani et al., 2023); (Miravet-Verde et al., 2019); (Delcourt et al., 2018). Conventional bottom-up mass spectrometry reinforced this bias: short, hydrophobic, low-abundance proteins yield few tryptic peptides, elute poorly, and are easily masked by abundant proteome constituents (Ahrens et al., 2021); (Fijalkowski et al., 2022) (Wacholder & Carvunis, 2023); (Miravet-Verde et al., 2019). Consequently, early MS datasets appeared to confirm the dogma that small translation events were rare or nonfunctional, supporting the notion that most smORF translation detected by transcriptomics or early Ribo-seq represented "noise" (Ahrens et al., 2021); (Wacholder & Carvunis, 2023); (Chothani et al., 2023); (Loaiciga et al., 2025).

The past decade has decisively overturned this view. Ribosome profiling (Ribo-seq), including specialized variants that trap initiating ribosomes, can map translated regions independent of ORF length and has uncovered thousands of actively translated smORFs across bacteria, yeast, animals, and plants (Weaver et al., 2019); (Chothani et al., 2023); (Schlesinger & Elsässer, 2021); (N. Li et al., 2025) (Y. Li et al., 2021). In parallel, proteomics optimized for small proteins, using tailored extraction, digestion, chromatography, and search strategies, has validated subsets of these translation events and revealed systematic blind spots in conventional workflows (Ahrens et al., 2021); (Hadjeras et al., 2023); (De Souza et al., 2024); (Fijalkowski et al., 2022); (Wang et al., 2020). Large integrative resources such as SmProt now catalog hundreds of thousands of small proteins across species, many derived from regions previously annotated as non-coding (Y. Li et al., 2021). Collectively, these advances establish that the "small proteome" is extensive, regulated, and biochemically stable enough to support function (Y. Li et al., 2021); (Ahrens et al., 2021); (Schlesinger & Elsässer, 2021); (Sandmann et al., 2023); (Wang et al., 2020).

This emerging functional picture aligns closely with the demands of cellular stress adaptation. Small proteins can be translated rapidly from short coding regions, often from pre-existing transcripts, enabling swift proteomic remodeling on timescales that match sudden heat, oxidative, or nutrient stress. Many are rapidly turned over, allowing cells to implement transient, reversible regulatory states without large biosynthetic investment (Schlesinger & Elsässer, 2021); (Wacholder & Carvunis, 2023); (Valdivia-Francia & Sendoel, 2024); (Loaiciga et al., 2025). In bacteria and archaea, small proteins are enriched at membranes and within transport and signaling modules, fine-tuning energy metabolism, envelope composition, and antibiotic resistance—key determinants of survival under stress (Jordan et al., 2023); (Miravet-Verde et al., 2019); (Hadjeras et al., 2023). In eukaryotes, microproteins emerge as components or regulators of core complexes, including mitochondrial and ER machineries, placing them at the center of unfolded protein responses, organelle stress, and quality control (Schlesinger & Elsässer, 2021); (Jordan et al., 2023); (Sandmann et al., 2023).

Mechanistically, small proteins often act as interaction modules rather than standalone enzymes. They bind and modulate larger proteins or complexes, alter the assembly or conformation of macromolecular machines, and serve as membrane anchors or adaptors (Jordan et al., 2023); (Schlesinger & Elsässer, 2021); (Miravet-Verde et al., 2019); (Hadjeras et al., 2023). This "microprotein logic" is well suited for rewiring stress networks: small proteins can be inserted into pre-existing pathways to adjust thresholds, switch outputs, or bridge signaling systems, with minimal structural footprint but significant regulatory impact. Ribosome-associated smORFs and uORFs further connect translational control to stress, as uORF translation can gate ribosome access to downstream ORFs and is dynamically reprogrammed during stress to prioritize protective gene expression (Valdivia-Francia & Sendoel, 2024); (Chothani et al., 2023).

Despite their late recognition, small proteins are widespread across all domains of life. Systematic Ribo-seq and peptidomics in bacteria, archaea, yeast, plants, and animals collectively indicate that a substantial fraction of each proteome consists of less or equal to 100-aa proteins (Y. Li et al., 2021); (Jordan et al., 2023); (Miravet-Verde et al., 2019); (Hadjeras et al., 2023); (Wang et al., 2020). Many smORFs show clear evolutionary conservation and signatures of purifying selection, comparable to longer canonical proteins, underscoring their biological relevance (Y. Li et al., 2021); (Sandmann et al., 2023); (Chothani et al., 2023); (Loaiciga et al., 2025). At the same time, phylostratigraphic analyses reveal that a large share of microproteins are evolutionarily young, arising de novo from non-coding regions and often maintained despite short length and low expression (Sandmann et al., 2023); (Chothani et al., 2023); (Loaiciga et al., 2025). This duality, deeply conserved cores alongside rapidly evolving, lineage-specific repertoires, suggests that small proteins provide both stability and flexibility: conserved modules sustain essential stress functions, while newly born microproteins can be quickly integrated to refine stress responses in specific ecological or developmental contexts.

Classical views of stress responses, heat shock systems, unfolded protein response, oxidative defenses, DNA damage checkpoints, nutrient starvation signaling, have largely emphasized canonical transcription factors, chaperones, and enzymes. Yet multiple lines of evidence now indicate that small proteins are interwoven into these same pathways. In bacteria, sORF-encoded polypeptides participate in envelope stress, stationary-phase adaptation, virulence, and

sporulation, and some are among the most essential genomic elements in minimal genomes (Jordan et al., 2023); (Miravet-Verde et al., 2019). In yeast and human cells, Ribo-seq reveals smORFs whose translation is specifically induced or repressed by stress, including those involved.

1.1 Definition, nomenclature, and genomic prevalence

Small open reading frames (smORFs/sORFs) are generally defined as coding sequences of ≤100 codons, a definition that was historically used in genome annotation to separate "true" coding regions from ORF-like sequences that are likely to be without function (Schlesinger & Elsässer, 2021); (Tong et al., 2024). In many bacterial and proteogenomic studies, however, even stricter limits of ≤50 amino acids are used, which reflects both the ubiquity of such ORFs and the difficulties of proving the existence of very small hydrophobic peptides with current experimental techniques (Weaver et al., 2019); (Hadjeras et al., 2023). Small open reading frames are ubiquitous; millions of potential smORFs are predicted in metazoan genomes, and a significant proportion of the proteins produced by bacteria also have fewer than 100 amino acids (Schlesinger & Elsässer, 2021); (Miravet-Verde et al., 2019); (Tong et al., 2024).

Terminology in the field distinguishes between the genomic elements and their translation products. The DNA or RNA elements are usually called smORFs/sORFs, while the proteins that are encoded by them are called microproteins, sORF-encoded proteins (SEPs), or sORF-encoded peptides (sPEPs) (Leong et al., 2022); (Dong et al., 2023). In eukaryotes, smORFs are often located in annotated non-coding regions, such as long noncoding RNAs, primary miRNAs, and circular RNAs (Y. Li et al., 2021); (Dong et al., 2023). The most common type of smORF, however, is an upstream ORF (uORF), which is located in the 5′ untranslated region (UTR) of a mRNA and can encode a peptide while also regulating the scanning of the ribosome by downstream ORFs (Y. Li et al., 2021); (Chothani et al., 2022). Variants in genes that create or abolish uORFs are strongly negatively selected, which indicates that their regulatory functions are essential (Y. Li et al., 2021).

Systematic searches for smORFs have shown that the number of translated smORFs is numerically equivalent to or greater than that of canonical ORFs. High-resolution Ribo-seq analysis in human tissues identified 7,767 translated smORFs with cell-type-specific expression and evolutionary conservation levels that are comparable to those of longer proteins (Chothani et al., 2022). In bacteria, integrated genomics suggests that ~16 ± 9% of all proteins per genome are ≤100 aa SEPs (Miravet-Verde et al., 2019). A large-scale resource, however, has confirmed that small proteins are ubiquitous features of proteomes: The SmProt database currently catalogs 638,958 unique small proteins (<100 aa) that are expressed in eight species, and this number is likely to be a small fraction of the true number of expressed small proteins (Y. Li et al., 2021).

1.2 Technological breakthroughs enabling discovery

The re-evaluation of small proteins has been made possible by Ribosome profiling (Ribo-seq) and optimized proteomics. The Ribo-seq technique involves sequencing ribosome-protected mRNA fragments to empirically identify translated regions without bias towards the length of ORFs; the technique has overcome the ≥100 codon bias of earlier annotation methods (Chothani et al., 2022); (Tong et al., 2024). Variants of the Ribo-seq method that stall the initiation of ribosomes at start codons enrich for short newly initiated ORFs, which enables the detection of a high number of smORFs and reveals hundreds of new small proteins in bacteria and human cells (Weaver et al., 2019); (Stringer et al., 2021). Comparative benchmarking studies show that the detection of smORFs is critically dependent on high-quality Ribo-seq data and that careful choice of analysis tool is required; thus, multiple algorithms (e.g., RibORF, RiboCode, ORFquant, Ribo-TISH, smORFer) should be used to avoid missing true ORFs (Tong et al., 2024); (Gelhausen et al., 2021); (Bartholomäus et al., 2020).

Optimized proteomics provides orthogonal evidence for the existence of small proteins, but specific protocols must be used to detect them because standard bottom-up proteomics methods are not effective for the analysis of small proteins: They are low in mass, hydrophobic, and often present in low abundance (Leong et al., 2022); (Miravet-Verde et al., 2019); (Hadjeras et al., 2023). Proteogenomic pipelines that use custom ORF databases for searches and Ribo-seq-informed search spaces (e.g., Rp3, iPtgxDB-based approaches) increase the confidence of detecting microproteins that are normally not visible in standard searches (De Souza et al., 2024); (Hadjeras et al., 2023).

Finally, resources and catalogues that consolidate the discoveries have been established. The SmProt database currently contains ~600,000 small proteins that are expressed in human cells, as well as ~10,000 small proteins that are encoded by uORFs in mRNAs of different species; these resources identify non-AUG initiation sites, associations with disease states, and uORF-mediated regulation of protein synthesis (Y. Li et al., 2021). Other reference sets have been generated from large-scale Ribo-seq compendia and curated smORF atlases in human and model organisms. These sets can be used as community standards for the annotation of the "small proteome" (Chothani et al., 2022); (Chothani et al., 2023); (Martinez et al., 2019). Together, these new methods and resources have transformed smORFs and their products from a

potentially noise population noise phenotype into a systematically mappable and experimentally tractable subset of the stress-responsive proteome.

1.3 Key concepts and enabling methods

Table 1 below summarized the concept and enabling methods

Table 1: Key concepts and enabling methods

Concept/Method	Role for small proteins	Reference
smORF/sORF (≤100 codons) definition	Formal size cutoffs; millions genome-wide	(Schlesinger & Elsässer, 2021); (Tong et al., 2024); (Leong et al., 2022)
SEPs / microproteins / sPEPs	Names for encoded small proteins	(Leong et al., 2022); (Miravet-Verde et al., 2019); (Dong et al., 2023)
uORFs in 5′ leaders	Encode peptides; regulate downstream translation	(Y. Li et al., 2021); (Chothani et al., 2023); (Martinez et al., 2019)
Ribo-seq (incl. initiation-stalling)	Length-independent detection of translated smORFs	(Chothani et al., 2023); (Chothani et al., 2022); (Weaver et al., 2019); (Stringer et al., 2021)
Specialized proteogenomics (Rp3, iPtgxDB)	Confirms SEPs; overcomes MS blind spots	(Miravet-Verde et al., 2019); (De Souza et al., 2024); (Hadjeras et al., 2023)
SmProt and related atlases	Catalog hundreds of thousands of small proteins	(Y. Li et al., 2021); (Chothani et al., 2023); (Martinez et al., 2019)

1.4 Identification and Characterization of Stress-Induced Small Proteins

Stress-induced small proteins are discovered by high-resolution ribosome profiling and functionally characterized by genetics and proteomics. These approaches show selective translation of smORFs under nutrient, heat, and oxidative stress and quantify their levels and activities.

1.5 Ribosome profiling and Ribo-RET for smORF discovery

Standard ribosome profiling (Ribo-seq) maps ribosome-protected RNA fragments across the genome to detect translation and defines ORF length independence of changes in expression that may occur due to the small size of smORFs (Weaver et al., 2019); (Vellappan, Sun, Favate, Jagadeesan, Cerda, & Yadavalli, 2025); (Pietras et al., 2024). In bacteria, combined elongation-stalling and Ribo-RET/Ribo-init approaches use antibiotics such as retapamulin or Onc112 to enrich 70S ribosomes that are trapped at start codons and provide high yield of initiating ribosomes that reveal many small proteins that are stress response factors and which would not be detected in proteomics analyses (Weaver et al., 2019). The resulting data are combined with RNA-seq and transcriptional reporter fusions and targeted tagging approaches to confirm expression (Vellappan, Sun, Favate, Jagadeesan, Cerda, & Yadavalli, 2025); (Weaver et al., 2019).

1.6 Accumulation under nutrient starvation and Mg^{2+} limitation

Nutrient starvation induces a specific set of small proteins. Under low Mg^{2+} conditions, ribosome profiling shows that E. coli has 17 small proteins (≤50 aa) that are upregulated in response to Mg^{2+} starvation (Vellappan, Sun, Favate, Jagadeesan, Cerda, & Yadavalli, 2025). Most of these small proteins are transcribedally activated through the PhoQ–PhoP two-component system that is the primary regulator of Mg^{2+} homeostasis in E. coli. Deletion of individual smORFs causes growth defects and results in altered cell sizes in low Mg^{2+} conditions, showing that these small proteins do have clear functions in the adaptation of E. coli to low Mg^{2+} conditions (Vellappan, Sun, Favate, Jagadeesan, Cerda, & Yadavalli, 2025). However, one small transmembrane protein, YoaI, accumulates in response to Mg^{2+} limitation even in the absence of the PhoQ–PhoP two-component system, and it is only activated through the phosphate responsive PhoR–PhoB two-component system. YoaI activates EnvZ–OmpR osmoregulatory signaling, showing that starvation-induced small proteins can cross-talk different stress response pathways (Vellappan, Sun, Favate, Jagadeesan, Cerda, & Yadavalli, 2025). Global proteomic studies on bacteria and mycobacteria have shown that nutrient limitation causes widespread remodelling of stress response modules in the proteome, including toxin–antitoxin pairs and proteases, although specific smORFs escape detection in these studies without Ribo-seq-guided searches (Albrethsen et al., 2013); (Yeom & Groisman, 2021).

1.7 Heat shock and oxidative stress-induced small proteins

Heat and oxidative stress cause the induction of small heat shock proteins (sHSPs; ~12–40 kDa) that act as ATP independent chaperones that prevent the irreversible aggregation of damaged proteins (Singh et al., 2024); (Banzet et al., 1998); (Wu et al., 2022). In plants, exposure to oxidative treatments such as H_2O_2, γ-irradiation and superoxide generated by chloroplasts causes the accumulation of sHSPs in chloroplasts and mitochondria. Different treatments induce different sHSPs with expression patterns that overlap but are not identical to classical heat shock responses (Banzet et al., 1998);

(Scarpeci et al., 2008); (Lee et al., 2000). sHSPs are associated with acquired thermotolerance and protection against oxidative damage, and their induction also confers cross protection against subsequent stresses (Lee et al., 2000). In animals, oxidative stress activates heat shock factors (HSFs) and additional transcription factors such as FOXO that coordinately upregulate both large HSPs and multiple sHSP genes to maintain proteostasis (Donovan & Marr, 2016); (Singh et al., 2024).

Although most work on heat and oxidative stress has focused on canonical sHSPs, Ribo-seq analyses increasingly reveal smORFs whose translation is selectively enhanced or repressed under these conditions, often in 5′ leaders or noncoding RNAs, suggesting a parallel layer of microprotein-mediated control that remains largely uncharacterized (Pietras et al., 2024); (Weaver et al., 2019); (Vellappan, Sun, Favate, Jagadeesan, Cerda, & Yadavalli, 2025).

1.8 Experimental pipelines for functional characterization

After stress-induced small proteins are identified, their functions are examined using:
Chromosomal tagging and immunoblotting to show that target proteins are induced at the protein level under specified stress conditions (e.g. Mg^{2+} withdrawal, H_2O_2 exposure, heat shock) (Weaver et al., 2019); (Vellappan, Sun, Favate, Jagadeesan, Cerda, & Yadavalli, 2025); (Banzet et al., 1998). Loss- and gain-of-function genetics to link smORF perturbation with growth, morphology or survival under starvation or oxidative/heat stress conditions (Vellappan, Sun, Favate, Jagadeesan, Cerda, & Yadavalli, 2025); (Banzet et al., 1998); (Mackei et al., 2021). Signaling readouts such as reporter fusions or phospho-specific assays to check whether small proteins modulate two-component systems, FOXO/HSF or other signaling kinases (Vellappan, Sun, Favate, Jagadeesan, Cerda, & Yadavalli, 2025); (Donovan & Marr, 2016); (Crawford et al., 2021). In bacteria, these approaches have established small membrane proteins like YoaI as signal integration nodes, while in plants and animals, stress-induced sHSPs and candidate microproteins are emerging as key components of chaperone and redox pathways (Vellappan, Sun, Favate, Jagadeesan, Cerda, & Yadavalli, 2025); (Donovan & Marr, 2016); (Banzet et al., 1998); (Wu et al., 2022); (Scarpeci et al., 2008).

Table 2: Representative methods and stress contexts

Aspect	Example / Role	References
Ribo-RET / initiation-stall profiling	Maps smORF start sites and stress-responsive initiation in E. coli	(Weaver et al., 2019); (Vellappan, Sun, Favate, Jagadeesan, Cerda, & Yadavalli, 2025)
Ribo-seq + RNA-seq under low Mg^{2+}	Identifies 17 Mg^{2+}-starvation-induced small proteins; PhoQ–PhoP and PhoR–PhoB control	(Vellappan, Sun, Favate, Jagadeesan, Cerda, & Yadavalli, 2025)
Small transmembrane protein YoaI	Accumulates in Mg^{2+} limitation; activates EnvZ–OmpR osmotic signaling	(Vellappan, Sun, Favate, Jagadeesan, Cerda, & Yadavalli, 2025)
Oxidative-inducible mitochondrial / chloroplast sHSPs	HSP22 in tomato, Oshsp26 in rice protect organelles against ROS and heat	(Banzet et al., 1998); (Lee et al., 2000); (Scarpeci et al., 2008)
FOXO-dependent sHSP and Hsp70 induction	Coordinates large and small HSP expression during oxidative stress in Drosophila	(Donovan & Marr, 2016)

1.9 Mechanisms of Action: Regulation of Signaling Networks

Small stress-induced proteins function as allosteric modulators of larger, membrane-based signaling complexes, rather than as enzymes. Through their interactions with histidine kinases, transporters, and the membrane itself, they reorganize stress signaling networks and alter their dynamics and connectivity.

1.9.1 Small proteins as stabilizers or modifiers of large targets

Bacterial small proteins generally interact with larger partners and modify their behavior. They can protect transporters from degradation (e.g., MgtS prevents the degradation of the Mg^{2+} transporter MgtA, while (Yadavalli et al., 2020); (Burton et al., 2024), or can target virulence factors for degradation (e.g., MgtR degrades the MgtC protein; (Yadavalli et al., 2020), thereby controlling the fluxes through metal homeostasis and virulence pathways (Yadavalli et al., 2020); (Burton et al., 2024). Other small proteins modify activity without catalysis: MgrB binds to the histidine kinase PhoQ and causes conformational changes that repress its activity (Jiang et al., 2023); (Yadavalli et al., 2020); (Salazar et al., 2016). More generally, small proteins often act as modules for protein–protein interactions, and modify the assembly, localization or stability of large complexes (Steinberg & Koch, 2021); (Burton et al., 2024).

1.9.2 Modulation of two-component systems

Two-component systems (TCSs) are preferred targets. In enterobacteria, various membrane microproteins are integrated into the TCS regulons, and interact with each other in feedback loops or connector loops:

MgrB (47 aa): induced by PhoP; binds to PhoQ, which represses its kinase activity but stimulates its phosphatase activity, producing partial adaptation of PhoP target genes after Mg^{2+} shift (Jiang et al., 2023); (Yadavalli et al., 2020);

B1500/SafA-like connector microprotein (65 aa): induced by EvgS–EvgA; directly interacts with PhoQ in the inner membrane and activates the PhoQ–PhoP TCS; links to Mg^{2+}/antimicrobial sensing and acid sensing (Eguchi et al., 2007); (Mao et al., 2025)

YoaI (27 aa): transmembrane smORF that is induced by PhoR–PhoB but accumulates in the absence of Mg^{2+}; activates EnvZ–OmpR, the osmotic-stress TCS, and mediates cross-talk between phosphate sensing, Mg^{2+} sensing, and osmotic sensing (Vellappan, Sun, Favate, Jagadeesan, Cerda, & Yadavalli, 2025)

The connector logic underlies the reprogramming of stress response hierarchies (Vellappan, Sun, Favate, Jagadeesan, Cerda, & Yadavalli, 2025); (Jiang et al., 2023); (Yadavalli et al., 2020); (Eguchi et al., 2007); (Mao et al., 2025)

1.9.3 Transmembrane small proteins and membrane stress

A large fraction of stress-induced small proteins are single-pass transmembrane proteins that interact with and remodel the lipid membrane. Mechanisms of action include:
Direct binding to membrane sensors: MgrB interacts with PhoQ in its TM region and periplasmic anchoring region, leading to changes in its orientation in membrane mini-domains that alter its sensitivity to antimicrobial peptides (Jiang et al., 2023).

Modifying membrane properties: Insertion of YohP (27 aa) proteins depolarizes the membrane, increases the levels of cardiolipin, decreases its fluidity and triggers accumulation of ppGpp, leading to a "metabolic silencing" program that enables cells to survive under adverse conditions (Natriashvili et al., 2025); (Steinberg & Koch, 2021)

Increasing stress resistance: ElaB, an inner membrane protein anchored via its C-terminal tail, increases resistance to oxidative and heat stress. Increased levels of this protein increases persister formation (Guo et al., 2017).

Conceptually, these transmembrane microproteins act as local rheostats at the lipid membrane, regulating signaling network components such as sensor kinase activity, efflux transporters, respiratory complexes, and membrane energetics (Yadavalli et al., 2020); (Steinberg & Koch, 2021); (Natriashvili et al., 2025); (Burton et al., 2024).

Table 3: Examples of signaling modulation by small membrane proteins

Small protein	Target / Pathway	Effect on signaling	References
MgrB (47 aa)	PhoQ–PhoP	Inhibits PhoQ kinase activity; establishes negative feedback and enables partial adaptation of the signaling pathway	(Jiang et al., 2023); (Yadavalli et al., 2020); (Salazar et al., 2016).
B1500 / SafA	EvgS–EvgA → PhoQ–PhoP	Activates PhoQ and links acid stress signaling to Mg^{2+} and antimicrobial peptide responses	(Eguchi et al., 2007); (Mao et al., 2025)
YoaI (27 aa)	EnvZ–OmpR	Activates the osmotic-stress two-component system and contributes to signaling cross-talk under Mg^{2+} limitation	(Vellappan, Sun, Favate, Jagadeesan, Cerda, & Yadavalli, 2025)
YohP (27 aa)	Membrane; stringent response	Depolarizes the membrane and induces (p)ppGpp accumulation, leading to metabolic downshift	(Steinberg & Koch, 2021); (Natriashvili et al., 2025)
ElaB	Inner membrane; RpoS-regulated	Enhances resistance to oxidative and heat stress while reducing cellular persistence	(Guo et al., 2017).

1.10 Small proteins in proteostasis: sHSPs, degradation pathways, and SUMO

Small proteins are central to protein quality control (PQC) by buffering misfolded proteins, routing them to refolding or degradation, and remodeling stress-response factors via SUMO modification.

1.10.1 Small heat-shock proteins (sHSPs) as chaperones

sHSPs are ATP-independent "holdase" chaperones (12–42 kDa) that bind early unfolding intermediates, preventing irreversible aggregation and keeping clients in a refolding-competent state (Reinle et al., 2021); (Janowska et al., 2019); (Mogk et al., 2019); (Treweek et al., 2014). They form large, dynamic oligomers that act as inactive reservoirs; stress

triggers oligomer remodeling, exposing binding sites and activating chaperone function (Reinle et al., 2021); (Janowska et al., 2019); (Mogk et al., 2019); (Peters et al., 2024). Bound substrates are sequestered into sHSP–client assemblies, which can then be handed off to Hsp70/Hsp100 systems for refolding, or routed to degradation (Reinle et al., 2021); (Albinhassan et al., 2025) Albinhassan et al., 2025; (Mogk et al., 2019); (Treweek et al., 2014). sHSPs are widely protective in neurodegeneration and aging by suppressing toxic aggregates (Albinhassan et al., 2025); (Hu et al., 2022); (Peters et al., 2024).

1.10.2 Coupling to proteasome and autophagy

PQC uses a triage: refold, sequester, or degrade. sHSP-built deposits (e.g., yeast Hsp42 foci) serve as sorting hubs that direct clients toward Hsp70-dependent refolding or selective autophagy (Reinle et al., 2021); (Mogk et al., 2019); When refolding fails, ubiquitin tagging targets substrates to the 26S proteasome or to autophagy; chaperones (Hsp70/Hsp90) help decide between these fates (Y. Li et al., 2022); (Marshall & Vierstra, 2019). Autophagy receptors such as p62/SQSTM1 bridge ubiquitinated aggregates to autophagosomes and also influence proteasome function, tightly coupling both degradation systems under proteotoxic stress (Liu et al., 2016); (Y. Li et al., 2022).

1.10.3 SUMOylation and modification of stress-response components

SUMO (a 97-aa ubiquitin-like modifier) is a small protein PTM that rapidly increases on thousands of targets during heat and proteotoxic stress, especially on proteins involved in folding, degradation, and nuclear quality control (Vertegaal, 2022); (Guo & Henley, 2014); (Cheng et al., 2023); (Sahin et al., 2022). SUMO chains on misfolded proteins recruit SUMO-targeted ubiquitin ligases (STUbLs) such as RNF4, promoting their ubiquitylation and proteasomal or autophagic clearance (Sahin et al., 2022). In plants and animals, stress-regulated SUMO conjugation and deconjugation fine-tune transcription, DNA repair, and nuclear condensates to maintain proteostasis under drought, salt, heat, and ischemic stress (Vertegaal, 2022); (Guo & Henley, 2014); (Sahin et al., 2022); (Ghimire et al., 2020); (Banda et al., 2025); (Augustine & Vierstra, 2018).

Table 1: Functional Roles of Small Proteins in Protein Quality Control (PQC)

PQC strategies involving small proteins	Mechanism	References
Holdase chaperone	Small heat shock proteins (sHSPs) bind misfolded client proteins and prevent their aggregation	(Reinle et al., 2021); (Janowska et al., 2019); (Mogk et al., 2019); (Treweek et al., 2014)
Sequestration hubs	sHSP foci organize misfolded proteins into compartments that facilitate triage toward refolding or autophagic degradation	(Reinle et al., 2021); (Mogk et al., 2019)
UPS vs autophagy decision	Chaperones together with ubiquitin E3 ligases determine whether damaged proteins are directed to the proteasome (UPS) or to autophagy pathways	(Liu et al., 2016); (Y. Li et al., 2022); (Marshall & Vierstra, 2019)

Small stress-responsive proteins are crucial for survival under stress, and their loss often causes specific growth defects and impaired local adaptation.

1.10.4 Loss-of-function phenotypes under stress

Across bacteria, yeast, and plants, the inactivation of stress-induced small proteins is generally without effect in unstressed conditions but causes extreme phenotypic defects upon the onset of stress, demonstrating their conditionally essential roles.

In E. coli, many small proteins (≤50 aa) only accumulate in response to specific types of stresses; their levels must be tightly regulated for proper growth and morphology only in those specific conditions (Hemm et al., 2009); (Yuan et al., 2025). For Mg^{2+} starvation in E. coli, deletion or overexpression of several small proteins regulated by the PhoQ/PhoP two-component system causes growth defects and changes in cell size under low Mg^{2+} conditions, demonstrating the conditionally important role of these proteins in adjusting to specific environmental conditions (Vellappan et al., 2025). The small membrane protein YohP causes sublethal depolarization of the cell membrane, condensation of the nucleoid, and decreased metabolic activity; the resulting “metabolic silencing” state protects cells from various types of damage, including antimicrobial peptides; thus any mis-regulation of this protein would impair survival in the face of envelope stress (Natriashvili et al., 2025); (Yuan et al., 2025). In plants, the small heat shock proteins (sHSPs) of mitochondria are also essential for proper growth and development in the absence of any applied stress; the triple knockdown of five different sHSP encoding genes in Arabidopsis thaliana causes severe phenotypic defects including chlorosis, growth inhibition, and decreased yield of seeds, as well as widespread reprogramming of photosynthetic and antioxidant enzyme

pathways (Escobar et al., 2021). Preventing the assembly of sHSP proteins into granules in tobacco plants causes cell death at even low sublethal levels of heat stress exposure (Miroshnichenko et al., 2004). Genome-wide expression analyses in plants have also revealed that many sHSP/small peptide encoding genes are expressed at very low baseline levels (or are nearly silent) in the absence of stress but are rapidly up-regulated within one hour upon exposure to heat, salt or plant hormones (Y. Li et al., 2022); (Wu et al., 2022); (Chang & Xiao, 2025); (Datta et al., 2024).

Context	Localized function of small proteins	References
Inner membrane (bacteria)	Small transmembrane proteins (e.g., YohP, YoaI) modulate membrane potential and regulate specific two-component signaling systems, enabling localized responses to Mg^{2+} limitation, osmotic stress, and envelope stress at the membrane	(Natriashvili et al., 2025); (Yuan et al., 2025). (Vellappan et al., 2025).
Mitochondria (plants)	Mitochondrial small heat shock proteins (M-sHSPs) stabilize mitochondrial proteins and membranes, indirectly influencing photosynthesis, ROS detoxification, and overall plant development	(Escobar et al., 2021)
Stress granules / cytosol (plants)	Cytosolic sHSPs assemble into heat-stress granules that sequester damaged proteins; disruption of this localized assembly can lead to membrane failure and cell death under moderate heat stress	(Miroshnichenko et al., 2004)
Apoplast / tissues (plants)	Small signaling peptides (<100 aa) act in specific tissues and extracellular spaces to regulate root growth, stomatal behavior, and other stress-adaptive physiological responses	(Wu et al., 2022); (Chang & Xiao, 2025); (Datta et al., 2024)

Future perspectives

Future work on small proteins will focus on improved purification and recovery methods and on multiscale models that link their low-molecular-weight properties to whole-cell behavior.

Overcoming purification and detection challenges

Small proteins (<~50 aa) are easily lost or obscured in standard protocols due to poor retention on columns, premature elution with the dye front, limited proteolytic fragments, and nonspecific binding to hydrophobic membrane proteins (Weaver et al., 2019). Strategies to overcome these issues include:

Modified MS protocols: No precipitation steps, optimized digestion, LC conditions, ionization parameters, and rigorous FDR analysis in project-specific databases allow for recovery of up to 99% of small proteins (Ahrens et al., 2021); (Weaver et al., 2019).

Top-down and nanopore methods: Intact small protein and proteoform analysis by top-down MS and emerging protein/nanopore analyses enable direct analysis without peptide coverage dependence (Ahrens et al., 2021); (Rukes & Cao, 2025).

Specific isolation technologies: Complexed diafiltration with amino acids keeps peptides in solution and minimizes aggregation (Elena et al., 2021); microfluidic nanobody-based affinity purification (μNANEX) automatically extracts sub-microgram amounts of tagged protein complexes from milliliter-scale lysates (De Keyser et al., 2024).

Trendy purification design: High-throughput screening and computational design of purification conditions is a strong trend in future purification design to minimize trial-and-error impact on active proteins (Du et al., 2022); (Tang et al., 2023); (Buyel, 2025).

Technological directions for system-level integration

Aim	Approach for Integrating Small Proteins	Sources
Augmenting Structure & Function	Utilizing homology models, structural proteomes (GEM-PRO), coevolutionary analysis, and deep learning to predict the 3D folds and binding interfaces of uncharacterized small proteins.	(Murray et al., 2021); (Brunk et al., 2016); (Braberg et al., 2022)
Embedding in Networks	Integrating small-protein datasets into genome-scale metabolic and regulatory networks, structural interactomes, and proteomic networks to establish physiological context.	(Murray et al., 2021); (Brunk et al., 2016); (Janes & Lauffenburger, 2006); (Liang et al., 2019)
Multi-scale Modeling	Coupling protein-level dynamics and allosteric models with network-level simulations to quantify the systemic impact of small regulatory proteins on cellular flux and stability.	(Murray et al., 2021); (Brunk et al., 2016); (Böde et al., 2007); (Liang et al., 2019)

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