Review

Loss of Proteostasis and Abnormal Aging in Down Syndrome: From Mechanisms to Interventions

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Loss of Proteostasis and Abnormal Aging in Down Syndrome: From Mechanisms to Interventions

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Abstract

Down syndrome (DS), caused by trisomy 21, is the most prevalent genetic condition associated with accelerated aging and near-universal development of early-onset Alzheimer's disease (AD). Beyond gene-dosage imbalance, trisomy 21 induces widespread transcriptional, metabolic, and proteomic remodeling that establishes a chronic state of proteotoxic and oxidative stress from early development. Increasing evidence identifies DS as a disorder of proteostasis network failure, in which sustained translational pressure, redox disequilibrium, and degradation pathway insufficiency progressively erode cellular resilience. In the DS brain, persistent endoplasmic reticulum stress with PERK-dominant signaling, mitochondrial dysfunction characterized by oxidative phosphorylation deficits and excessive reactive oxygen species production, and impaired antioxidant responses create a highly vulnerable intracellular environment. Concomitantly, degradation systems become compromised: proteasomal catalytic activity declines, ubiquitin-dependent signaling is remodeled, and chronic mTOR hyperactivation suppresses autophagic and mitophagic flux. The coordinated impairment of the ubiquitin–proteasome system and autophagy establish a feed-forward cycle of proteotoxic accumulation and redox amplification. Within this framework, Alzheimer-like neuropathology in DS emerges not solely from amyloid precursor protein triplication but as the late manifestation of decades-long proteostasis exhaustion. Therapeutic strategies aimed at restoring global proteostasis and redox balance may therefore represent a more effective systems-level approach to mitigating neurodegeneration in DS.

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1. Introduction

Down syndrome (DS), caused by full or partial trisomy of chromosome 21 (Chr21), is the most common chromosomal aneuploidy compatible with postnatal survival and the leading genetic cause of intellectual disability, affecting approximately 1 in 800–1,000 live births worldwide [1]. Over recent decades, advances in cardiac surgery, antimicrobial therapies, and endocrine management have markedly increased life expectancy, transforming DS into a lifespan condition. As survival has improved, it has become increasingly evident that DS is not merely a neurodevelopmental disorder, but a systemic and progressive condition characterized by premature aging and early emergence of age-associated comorbidities. Clinically, DS presents with intellectual disability and a broad constellation of multisystem alterations—including congenital heart defects, thyroid dysfunction, immune dysregulation, gastrointestinal anomalies, metabolic disturbances, and increased susceptibility to hematological malignancies. However, this phenotypic heterogeneity reflects a deeper biological principle: trisomy 21 does not simply increase the expression of individual genes but reshapes regulatory networks across the genome [2]. The additional chromosome imposes

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genome-wide transcriptional, epigenetic, and proteomic remodeling that alters developmental trajectories and systemic homeostasis from embryogenesis onward [3]. At the molecular level, overexpression of more than 200 Chr21 genes perturbs chromatin organization, modifies DNA methylation patterns, alters histone marks, and reshapes non-coding RNA networks [4]. These changes extend beyond proportional transcript amplification, affecting global gene expression programs involved in metabolism, immune signaling, mitochondrial function, and cellular stress responses. Consequently, trisomic cells operate in a state of persistent regulatory disequilibrium. Increased gene dosage elevates global protein synthesis and disrupts stoichiometric relationships within multiprotein complexes, creating a chronic imbalance between protein production, folding, and degradation. This sustained pressure on protein quality control systems establishes a proteotoxic background that intersects with redox imbalance and metabolic dysregulation [5]. The central nervous system (CNS) is particularly vulnerable to these disturbances. Neurodevelopment in DS is characterized by reduced neural progenitor proliferation, altered neuronal differentiation, impaired synaptogenesis, and diminished dendritic arborization, leading to reduced brain volume—especially within the hippocampus and frontal cortex. These structural abnormalities unfold within a cellular environment marked by mitochondrial dysfunction, increased oxidative stress, and chronic low-grade inflammation. Triplication of interferon receptor genes enhances innate immune responsiveness, while overexpression of redox-regulating genes such as SOD1 perturbs antioxidant balance [6]. The convergence of these mechanisms establishes a stress-prone neural milieu that predisposes to premature biological aging. Among the most consequential gene-dosage effects is triplication of the amyloid precursor protein (APP) gene, which confers an almost inevitable risk of early Alzheimer-related neuropathology [7]. Yet Alzheimer's disease (AD) in DS cannot be attributed solely to amyloid overproduction. Amyloid-β accumulation unfolds within a broader landscape of progressive mitochondrial fragility, impaired proteostasis, chronic oxidative stress, and diminished adaptive stress responses. Thus, AD pathology in DS emerges not as an isolated consequence of APP dosage [8], but as the downstream manifestation of long-standing cellular disequilibrium.

Individuals with DS represent the most prevalent genetically determined form of early-onset Alzheimer's disease (EOAD), with nearly universal development of AD neuropathology by the fourth decade of life [9]. However, amyloid accumulation in DS occurs within a biological context of systemic and brain-specific accelerated aging. Trisomy 21 establishes a persistently stress-prone cellular state decades before chronological aging would predict, lowering resilience thresholds and amplifying the neurotoxic effects of amyloid-β (Aβ) and tau pathology. Beyond APP triplication, overexpression of Chr21 genes such as SOD1, DYRK1A, and RCAN1 contributes to dysregulation of redox signaling, kinase activity, mitochondrial function, and calcium homeostasis [10]. These alterations promote sustained oxidative stress, metabolic instability, and impaired stress adaptation. Mitochondrial dysfunction emerges as a central vulnerability node, characterized by impaired oxidative phosphorylation, altered mitochondrial dynamics, defective mitophagy, and excessive production of reactive oxygen species (ROS) [11]. Bioenergetic insufficiency not only compromises neuronal function but also weakens energy-dependent proteostasis mechanisms, reinforcing a feed-forward cycle between redox imbalance and proteotoxic stress. Persistent oxidative and metabolic stress promotes premature activation of cellular senescence programs across neurons and glial populations. Senescent cells exhibit activation of p53/p21 and p16^INK4a^ pathways, chronic DNA damage signaling, mitochondrial impairment, and extensive epigenetic remodeling [12]. A hallmark of senescence is progressive loss of proteostasis capacity, including impaired autophagic flux, reduced proteasomal activity, and maladaptive unfolded protein responses [13]. In this context, mitochondrial dysfunction limits ATP availability for protein folding and degradation, while oxidative modifications increase the aggregation propensity of Aβ and tau. The senescence-associated secretory phenotype (SASP) further sustains inflammatory signaling and activates tau kinases, directly linking premature cellular aging to synaptic dysfunction and network instability [14]. Neuropathologically, AD in DS mirrors sporadic AD in its anatomical progression but develops decades earlier. Amyloid deposition begins in early adulthood, followed by widespread tau

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pathology by the fourth decade, whereas clinical dementia manifests later as compensatory mechanisms progressively fail [15]. Biomarker studies—including cerebrospinal fluid analyses and PET imaging—demonstrate a prolonged preclinical phase during which mitochondrial dysfunction, oxidative stress, and proteostatic insufficiency silently accumulate. This predictable and genetically defined trajectory positions DS as a powerful human model for dissecting the interplay between accelerated aging and neurodegeneration [16].

2. Trisomy 21 and Proteotoxic Stress

Aneuploidy imposes intrinsic stress on cellular systems, and trisomy 21 exemplifies this principle [17]. Increased gene dosage elevates global protein synthesis and disrupts the stoichiometric balance required for correct assembly of multiprotein complexes. Proteins encoded on chromosome 21 frequently require binding partners located on other chromosomes; when produced in excess, they remain unassembled or misfolded, increasing the burden on molecular chaperones and degradation pathways [18]. This imbalance establishes a chronic proteostatic strain in which stress-response pathways remain persistently engaged. Proteotoxic stress in DS is further amplified by mitochondrial dysfunction and redox imbalance. Oxidative modifications destabilize protein structure and promote aggregation, while impaired ATP production compromises the efficiency of energy-dependent clearance systems, including the ubiquitin–proteasome system and autophagy–lysosome pathways. Rather than reflecting isolated pathway failures, proteostasis disruption in DS represents a network-level disturbance in which increased protein synthesis, defective degradation, oxidative stress, altered translational control, and organelle dysfunction converge [19]. Neurons are particularly susceptible to this chronic imbalance due to their high metabolic demand, complex proteome dynamics, and limited regenerative capacity. Over time, sustained proteostatic insufficiency reduces synaptic protein turnover, weakens adaptive stress responses, and lowers the threshold for aggregation-driven toxicity [20]. Aging superimposed on this pre-stressed background further diminishes buffering capacity, accelerating the transition from compensatory stress signaling to irreversible neurodegeneration. Viewed through this integrative lens, DS emerges as a paradigmatic condition in which gene-dosage imbalance drives chronic proteotoxic stress, accelerates biological aging, and creates a permissive environment for Alzheimer-like pathology. The following sections will dissect how endoplasmic reticulum stress, mitochondrial dysfunction, and degradation pathway impairment interact within a unified proteostasis–redox network and will explore therapeutic strategies aimed at restoring systemic resilience rather than targeting single pathogenic proteins.

2.1. Endoplasmic Reticulum Stress and Unfolded Protein Response in Down Syndrome Neuropathology

Trisomy 21 imposes a chronic proteotoxic burden that persistently engages endoplasmic reticulum (ER) stress signaling and reshapes unfolded protein response (UPR) dynamics in DS. Rather than representing a transient adaptive reaction, ER stress in DS appears developmentally established and progressively maladaptive. The ER, a central hub for protein folding, calcium homeostasis, and post-translational modification, is highly sensitive to disturbances in redox balance and proteome load. In trisomic cells, increased protein synthesis, oxidative modifications, and altered calcium handling converge to destabilize ER proteostasis, promoting accumulation of misfolded proteins and sustained activation of UPR pathways [21,22] . Although the UPR is classically defined as a conserved adaptive program that reduces translation and enhances folding capacity [23], its chronic engagement can shift from protective to deleterious, ultimately promoting apoptosis and functional decline [24]. Multiple independent lines of evidence indicate that ER stress and maladaptive UPR activation are embedded features of DS neuropathology. Proteomic and biochemical analyses of human DS brain tissue demonstrate increased activation of ER stress sensors and dysfunction of key ER chaperones [25–27]. Accumulation of oxidatively modified proteins within the ER lumen activates the three canonical UPR branches—PERK, ATF6, and IRE1—via dissociation from the master regulator GRP78/BiP [22]. Notably, GRP78 itself is oxidatively modified in DS brain [19], impairing its regulatory capacity and providing a direct mechanistic link between redox

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imbalance and sustained ER stress signaling [28]. Among UPR branches, the PERK pathway emerges as the dominant and persistently engaged arm in DS. Biochemical studies of frontal cortex from individuals with DS reveal chronic activation of the PERK/eIF2α/ATF4 axis [26]. PERK-mediated phosphorylation of eIF2α reduces global protein synthesis, thereby limiting ER client load [29], while selectively enhancing translation of stress-responsive transcripts such as ATF4 [30]. Under physiological conditions, ATF4 coordinates adaptive outputs, including induction of chaperones, redox-regulatory genes, autophagy components, and amino acid metabolism pathways. Negative feedback through GADD34 allows re-establishment of translational homeostasis [31]. In DS, however, this regulatory balance appears disrupted. Reduced GADD34 levels and persistent eIF2α phosphorylation indicate failure to restore translational equilibrium, leading to sustained activation of pro-apoptotic mediators such as CHOP and BCL-2 family members [32]. Importantly, PERK hyperactivation in DS is closely associated with Alzheimer-like neuropathological features. Both young and older DS brains exhibiting AD pathology show increased pPERK, peIF2α, ATF4, and CHOP levels [26], paralleling observations in sporadic AD, where ER stress markers correlate with Braak stage progression [26,33–36]. These findings suggest that PERK-dominant UPR signaling is not a secondary epiphenomenon but a central contributor to neurodegenerative vulnerability. A critical pathogenic feature in DS is the uncoupling between PERK activation and antioxidant defense. Under normal conditions, PERK signaling promotes nuclear translocation of Nrf2, enhancing antioxidant gene expression. In DS, despite sustained PERK activation, Nrf2-driven transcription is suppressed [26]. This maladaptive dissociation is linked to overexpression of Bach1, a chromosome 21–encoded transcriptional repressor of Nrf2 targets [37,38], resulting in continued translational repression without adequate redox compensation. Consequently, the DS brain exhibits a state of PERK dominance combined with antioxidant insufficiency, amplifying oxidative damage and reinforcing proteostatic imbalance [39].

Systems-level proteomic analyses further support the centrality of ER stress in DS. Large-scale studies of post-mortem frontal cortex from young and aged individuals with DS reveal early and persistent dysregulation of stress-response and proteostasis pathways that intensify with aging and AD conversion [40–43]. Importantly, these alterations are detectable before overt neurodegeneration, indicating that UPR dysregulation is embedded within the DS molecular phenotype rather than representing a late-stage response. Energy metabolism, synaptic transmission, and cellular stress-response networks consistently emerge as the most affected pathway clusters, reinforcing the concept that chronic ER stress contributes to progressive neuronal dysfunction [40]. Proteostasis abnormalities in DS are not restricted to the CNS. Proteomic profiling of peripheral blood mononuclear cells (PBMCs) from children with DS demonstrates widespread dysregulation of metabolic, trafficking, and stress-response pathways, including activation of ER stress and UPR components [26,44]. Increased oxidative protein damage and sustained UPR signatures are detectable early in life, supporting the concept of a developmentally established “pre-stressed” proteostatic state. Similarly, lymphoblastoid cell lines derived from children with DS display persistent UPR induction and early endosomal alterations, consistent with systemic proteotoxic burden [26,45]. Broader analyses of the proteostasis network in DS cells confirm coordinated changes in chaperone systems, UPR mediators, and proteasomal machinery, alongside heightened sensitivity to additional proteotoxic challenges [18,41,44,46]. Reduced buffering capacity and lower stress tolerance thresholds suggest that trisomic cells operate closer to proteostasis collapse.

Murine models of DS provide temporal and mechanistic resolution of ER stress dynamics [47]. The Ts65Dn model exhibits early activation of ER stress pathways in the brain, with selective engagement of the PERK branch detectable prior to overt neurodegeneration [48]. Increased phosphorylation of PERK and eIF2α at young ages indicates that ER stress signaling is an upstream event in DS neuropathology. Longitudinal profiling reveals that UPR activation is not static; early hyperactivation may transition into partial signaling exhaustion or maladaptive remodeling with aging, consistent with progressive loss of stress-response efficiency [49,50]. This temporal evolution aligns with the broader concept of premature molecular aging in the DS brain.

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Mechanistic validation is provided by studies in the Ts2Cje model, where pharmacological inhibition of PERK signaling restores translational balance, enhances Nrf2 nuclear translocation, and corrects Nrf2/Bach1 imbalance [26]. These interventions demonstrate that PERK hyperactivation is not merely correlative but causally linked to oxidative and proteostatic dysfunction, highlighting its therapeutic tractability. The more recently developed Ts66Yah model, which improves construct validity by eliminating non-Hsa21 orthologous regions [51], confirms early and persistent dysregulation of stress-response and proteostasis pathways across the lifespan [52]. Although not restricted to ER markers, proteomic analyses consistently identify UPR components among the most perturbed molecular signatures, reinforcing the centrality of chronic stress-response engagement in DS neuropathology [40]. Age-dependent cognitive decline in Ts66Yah mice parallels progressive redox imbalance and proteostasis disruption, supporting the notion that ER stress contributes to long-term functional vulnerability.

Beyond the canonical UPR, the integrated stress response (ISR) is also activated in DS. Costa-Mattioli and colleagues demonstrated phosphorylation of eIF2α in Ts65Dn hippocampus, post-mortem DS brain tissue, and DS-derived induced pluripotent stem cells (iPSCs), mediated in part by PKR activation [27]. Importantly, normalization of eIF2α phosphorylation restored proteostatic balance and improved cognitive performance in DS models [27], underscoring the pathological relevance of sustained translational repression.

Collectively, convergent evidence from human brain, peripheral cells, and multiple DS mouse models positions ER stress and UPR dysregulation as central nodes in DS pathobiology. Trisomy 21 establishes chronic proteotoxic and oxidative pressure that persistently activates ER stress sensors, biases UPR signaling toward PERK dominance, uncouples antioxidant defense, and progressively undermines synaptic and neuronal integrity. While acute UPR activation may initially buffer proteome imbalance, sustained engagement—particularly of the PERK–ATF4–CHOP axis—promotes translational repression, pro-apoptotic signaling, and heightened vulnerability to Alzheimer-like neurodegeneration [53]. These findings collectively identify ER stress modulation, restoration of Nrf2 activity, and reinforcement of proteostasis capacity as rational therapeutic strategies to counteract neurodegenerative progression in Down syndrome [25,26,40].

3. Mitochondrial Stress in Down Syndrome

In DS, mitochondria emerge as a central bioenergetic and redox hub through which trisomy-driven proteotoxic pressure is translated into progressive cellular dysfunction. Beyond their canonical role in ATP production, mitochondria regulate calcium buffering, redox signaling, lipid metabolism, and apoptotic pathways—functions that are particularly critical in neurons and glial cells with high metabolic demand and limited regenerative capacity [54]. In trisomy 21, mitochondrial destabilization leads to increased production of reactive oxygen and nitrogen species (ROS/RNS), propagating oxidative modifications of proteins and lipids, including carbonylation, nitration, and peroxidation-derived adducts [55,56]. Persistent oxidative stress damages mitochondrial DNA (mtDNA), respiratory chain components, and membrane integrity [57], further impairing electron transport chain (ETC) efficiency and reducing ATP output. This bioenergetic decline not only compromises neuronal function but also destabilizes energy-dependent proteostasis systems, amplifying protein misfolding and aggregation under oxidizing conditions [58]. Mitochondria therefore act as a mechanistic bridge linking gene-dosage imbalance to accelerated aging and Alzheimer-like neurodegeneration in DS [55,56,58–60]. Hallmarks of mitochondrial dysfunction in DS include reduced oxidative phosphorylation (OXPHOS) efficiency, chronic redox imbalance, and disruption of mitochondrial quality control (MQC) programs encompassing biogenesis, dynamics, and mitophagy [28,61]. These alterations are not restricted to the central nervous system; they contribute to systemic phenotypes such as cognitive impairment, congenital cardiac defects, and premature aging traits [1,56,61]. Importantly, mitochondrial dysfunction is detectable early in development, with widespread abnormalities reported in fetal trisomy 21-derived cells [62–64]. Structural fragmentation of the mitochondrial network, cristae disorganization, and

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reduced connectivity indicate chronic imbalance in mitochondrial turnover and distribution [65]. During neurogenesis and synaptic maturation—periods of intense bioenergetic demand—such instability is particularly detrimental, correlating with impaired neuronal proliferation, altered differentiation, reduced dendritic complexity, and increased susceptibility to apoptosis in DS models [28,56,60,66–69].

3.1. Mitochondrial Unfolded Protein Response (UPRmt) in Down Syndrome

The mitochondrial unfolded protein response (UPRmt) constitutes a critical adaptive program that attempts to buffer mitochondrial proteotoxic stress by coordinating retrograde signaling to the nucleus [70]. Upon accumulation of misfolded proteins within the mitochondrial matrix, UPRmt induces transcriptional programs that enhance expression of mitochondrial chaperones, proteases, antioxidant defenses, and metabolic regulators [70–72]. This response aims to restore ETC integrity, stabilize protein folding, and prevent propagation of mitochondrial stress to the broader cellular environment.

In DS, however, UPRmt engagement appears early but functionally incomplete. In the Ts2Cje model, perinatal activation of an ATF5/GRP75-dependent program in frontal cortex is accompanied by reduced expression of other stress mediators, including ATF4, CHOP, and SIRT3 [26,73,74]. This partial activation suggests that mitochondria in DS detect proteotoxic stress yet fail to mount a fully coordinated protective response. Such imbalance may limit stabilization of respiratory chain complexes and compromise mitochondrial proteome maintenance under sustained oxidative conditions [1]. Consequently, incomplete UPRmt engagement may contribute to persistent mitochondrial fragility during neurodevelopmental windows when bioenergetic precision is essential.

When UPRmt buffering proves insufficient, cells rely increasingly on broader MQC mechanisms—including biogenesis, fusion–fission dynamics, and mitophagy—to maintain a functional mitochondrial pool [73]. In DS, evidence indicates that these compensatory systems are chronically stressed and frequently maladaptive, favoring accumulation rather than efficient turnover of dysfunctional organelles.

Impaired OXPHOS is a consistent and defining feature of DS biology. Across human tissues, patient-derived cells, and mouse models, trisomy 21 is associated with ETC inefficiency, particularly involving complex I deficits, reduced bioenergetic reserve, and increased ROS generation [56,60,68,74–78]. Some evidence suggests that trisomic cells downregulate OXPHOS as an adaptive attempt to limit electron leak and oxidative damage [79]. While this strategy may transiently reduce ROS production, it compromises ATP availability and diminishes metabolic flexibility, particularly under fluctuating energy demands.

In the developing and adult brain—where synaptic plasticity and circuit refinement require rapid, localized ATP production—chronic OXPHOS dampening may have profound consequences for neuronal resilience. In Ts2Cje frontal cortex, dysfunction of complexes I and IV emerges early during developmental stages characterized by maximal synaptogenesis [74]. Moreover, lifespan-dependent remodeling of OXPHOS subunits—where some components are upregulated and others downregulated—suggests instability in ETC assembly and turnover under persistent proteostatic pressure [52,74]. Proteomic profiling of human DS frontal cortex with and without AD neuropathology consistently identifies mitochondrial and energy-related pathways among the most altered signatures [40], underscoring the centrality of bioenergetic stress across DS and DSAD trajectories. Emerging evidence also implicates complex IV as a metabolic bottleneck, with upstream metabolic inhibitors converging on cytochrome c oxidase function [80].

ETC complexes are multi-subunit structures requiring coordinated nuclear and mitochondrial gene expression, import, folding, and assembly. Under chronic proteotoxic and oxidative stress, this coordination becomes destabilized. Thus, OXPHOS inefficiency in DS is both a consequence and an amplifier of mitochondrial proteostasis failure, reinforcing a feed-forward loop of ROS production, energetic insufficiency, and progressive proteostatic collapse in the brain.

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3.2. Mitochondrial Quality Control (MQC) in Down Syndrome

MQC integrates mitochondrial biogenesis, fusion–fission dynamics, and mitophagy to preserve organelle integrity under stress [81] In DS, converging data indicate that MQC is persistently challenged, particularly in neural tissue where spatially restricted ATP production and tight redox regulation are critical [55,56]. Morphologically, fragmentation of the mitochondrial network and cristae disorganization reflect imbalance in fusion–fission regulation [55,78,82]. Reduced fusion competence (MFN2/OPA1 dysfunction and altered OPA1 processing) combined with enhanced DRP1-mediated fission promotes respiratory inefficiency and ROS amplification [55,74,77,83,84]. Notably, experimental normalization of mitochondrial dynamics improves bioenergetics and supports neurogenic programs in DS models, confirming that dynamics are functionally causal rather than epiphenomenal [75,83–87]. Mitophagy impairment further exacerbates mitochondrial accumulation. Evidence of defective PINK1/Parkin signaling, incomplete autophagic flux, and inefficient removal of damaged mitochondria indicates compromised turnover capacity [74,88,89]. Persistent mTOR hyperactivation—reported early and sustained in DS brain—restrains ULK1-mediated initiation of autophagy and mitophagy [74,88–90], thereby linking nutrient-sensing dysregulation to defective mitochondrial clearance. ULK1-dependent phosphorylation of mitophagy receptors such as FUNDC1 [90] provides a mechanistic bridge between mTOR status and cargo recruitment, reinforcing the notion that mTOR dysregulation [59,91–94] directly impairs mitochondrial quality control. Simultaneously, dysregulation of mitonuclear signaling pathways—including AMPK–PGC-1α–NRF1–TFAM—limits mitochondrial renewal and mtDNA maintenance under sustained stress [55,74,87]. Trisomy-linked gene dosage further compounds MQC dysfunction. Overexpression of ETS2 promotes mitochondrial apoptotic signaling in DS neuronal models [95], while intersectin-1 (ITSN1) influences mitochondrial death pathways via trafficking modules [96]. Repression of PGC-1α activity through DYRK1A/RCAN1–calcineurin/NFAT signaling [69,97,98] and NRIP1 overexpression [99] constrains mitochondrial biogenesis. Additionally, HSA21-encoded microRNAs, such as miR-155-5p targeting TFAM [100] and let-7c-5p potentially targeting ANT1/SLC25A4 [101], further destabilize mitochondrial maintenance. Collectively, these trisomy-driven inputs create an imbalance wherein mitochondrial damage accumulates faster than MQC systems can compensate, accelerating redox stress and proteostatic vulnerability.

3.3. Insulin Resistance as a Proteostasis–Redox Switch in the DS Brain

In the CNS, insulin signaling functions as a metabolic rheostat that integrates nutrient sensing with mitochondrial competence, redox control, and proteostasis [102–105]. Through the IRS–PI3K–AKT cascade, insulin promotes metabolic flexibility, restrains excessive ROS generation, and modulates mTOR-dependent translation and autophagy pathways [102–105]. In DS, this insulin–mTOR axis is disrupted early and prominently in the brain [40,59,60,68,69,89,91]. Aberrant activation of PI3K/AKT/mTOR signaling has been documented in post-mortem DS tissue [88,89,91], reinforcing mTOR hyperactivation as a core trisomy-linked stress signature. Sustained mTOR activity suppresses ULK1-dependent autophagy initiation, impairs mitophagy, and favors persistence of ROS-generating mitochondria [88,89,91]. Brain insulin resistance (BIR) likely contributes upstream to this maladaptive state [59,60,68], linking metabolic signaling failure to mitochondrial dysfunction and oxidative damage. Neuronal-derived extracellular vesicles from young individuals with DS reveal coordinated insulin pathway disruption and aberrant mTOR activity [59], suggesting that this imbalance is developmentally established. O-GlcNAcylation adds an additional nutrient-sensitive regulatory layer. Reduced glucose uptake and insulin resistance decrease hexosamine biosynthetic pathway flux and UDP-GlcNAc availability, lowering protein O-GlcNAcylation and altering the balance between phosphorylation and adaptive stress signaling. In DS models, O-GlcNAc dysregulation coexists with OXPHOS defects and oxidative stress [106]. Taken together, these findings position insulin resistance not merely as a metabolic comorbidity but as a proteostasis–redox switch that shapes mitochondrial resilience and neurodegenerative vulnerability across the DS lifespan.

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4. Ubiquitin–Proteasome System Dysfunction in Down Syndrome Brain

The ubiquitin–proteasome system (UPS) is the principal intracellular machinery responsible for selective protein degradation and dynamic regulation of protein turnover. Beyond its degradative function, ubiquitin-dependent signaling orchestrates stress responses, apoptosis, transcriptional regulation, and metabolic adaptation, thereby functioning as a central regulator of proteostasis [107]. Protein targeting to the proteasome requires coordinated ubiquitin conjugation via E1, E2, and E3 enzymes, leading to polyubiquitin chain formation—most commonly through Lys48 linkages—recognized by the 26S proteasome, a multicatalytic complex endowed with chymotrypsin-like, trypsin-like, and caspase-like activities [108]. Oxidative modification of proteins can facilitate proteasomal recognition through partial unfolding, functionally linking redox balance to proteolytic efficiency [109]. In DS, converging evidence indicates progressive disruption of this finely tuned system. A landmark study by our research group provided the first comprehensive characterization of the polyubiquitinylation profile in DS frontal cortex before and after Alzheimer neuropathology. Isolation of polyubiquitinated proteins from postmortem samples revealed a profound age-dependent remodeling of the ubiquitome [110]. Proteins displaying aberrant ubiquitin tagging were predominantly involved in protein quality control and energy metabolism, and oxidative modifications were closely associated with altered ubiquitin conjugation patterns [110,111] . These findings demonstrate that ubiquitin signaling dysregulation represents an early molecular alteration accompanying DS brain aging rather than a late consequence of amyloid deposition [110]. Functional analyses further revealed significant reductions in proteasomal catalytic activities—including chymotrypsin-like, trypsin-like, and caspase-like activities in DS frontal cortex, supporting impaired substrate clearance during early disease stages [110]. Similar reductions in proteasomal activity and increased ubiquitin-positive aggregates have been reported in DS fibroblasts and in the cerebellum of Ts65Dn mice [19,112] , reinforcing the concept of systemic UPS vulnerability. Mechanistically, oxidative post-translational damage represents a major contributor to UPS dysfunction in DS. Redox proteomics analyses identified ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1), a neuron-enriched deubiquitinating enzyme essential for ubiquitin recycling, as an early target of oxidative modification in DS brain [19]. Subsequent studies demonstrated aberrant polyubiquitinylation of UCH-L1, suggesting structural instability and functional impairment [113]. Given its role in maintaining free ubiquitin pools, oxidative inactivation of UCH-L1 may further compromise proteasomal efficiency. Similar carbonylation-associated loss of UCH-L1 activity has been described in AD brain, reinforcing the pathogenic relevance of this mechanism [114]. Accumulation of polyubiquitinated proteins in DS cortex indicates that ubiquitinated substrates are not efficiently degraded and may remain stalled within the system. Increased oxidation of SOD1, a feature observed in DS brain, has been linked to inhibition of proteasome activity, further supporting direct interference of chronic redox imbalance with proteolytic capacity [111,114]. In addition to oxidative mechanisms, gene dosage effects inherent to trisomy 21 may contribute to UPS perturbation. Chromosome 21 encodes multiple UPS regulators, including deubiquitinating enzymes such as USP25 and USP16, as well as proteins involved in ubiquitin conjugation and proteasome assembly [115]. Although the direct functional consequences of their triplication remain incompletely defined, altered expression of UPS-related genes may destabilize ubiquitin homeostasis in DS brain. Furthermore, triplication of BACH1 perturbs the nuclear BACH1/NRF2 balance, impairing antioxidant responses and amplifying oxidative stress [37,38,116]. Given the sensitivity of proteasome function to redox status, BACH1 gene dosage represents an important indirect contributor to UPS vulnerability. Altogether, enzymatic impairment, oxidative damage, and gene dosage imbalance converge to compromise ubiquitin-dependent proteolysis in DS. As proteasomal degradation becomes progressively insufficient, cells increasingly rely on complementary degradative pathways particularly the autophagy lysosomal system to maintain proteostasis.

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5. Autophagy in Down Syndrome

Autophagy is a highly conserved degradation pathway responsible for the clearance of long-lived proteins, aggregates, and damaged organelles, including mitochondria and ER [117] . Through autophagosome formation and lysosomal fusion, autophagy ensures cytoplasmic quality control and metabolic adaptation under stress [118]. Its activity is tightly regulated by nutrient-sensitive signaling pathways, most prominently the mTOR axis [119]. In DS, accumulating evidence indicates profound and early autophagy dysregulation. Post-mortem DS frontal cortex exhibits hyperactivation of the PI3K/AKT/mTOR pathway associated with reduced autophagosome formation and altered expression of autophagy-related proteins [89]. Notably, mTORC1 hyperactivation is detectable during prenatal and early postnatal development in the DS hippocampus and persists across the lifespan [88], indicating chronic suppression of autophagic induction. These alterations correlate with increased Aβ accumulation and tau phosphorylation [120,121]**.** Experimental models reproduce these findings. Ts1Cje and Ts65Dn mice show reduced autophagic flux, altered LC3 processing, and dysregulation of Atg proteins, together with accumulation of pathogenic protein species [91,122,123] . Similarly, DS-derived fibroblasts display defective macroautophagy and impaired PINK1/PARKIN-dependent mitophagy [89]. Pharmacological inhibition of mTOR restores autophagic flux and partially rescues mitochondrial defects [93,124,125]. Additional trisomy-linked mechanisms further constrain autophagic efficiency. APP triplication and accumulation of its C99 fragment impair lysosomal acidification through interference with the V0-ATPase proton pump [126]. Oxidative modifications of autophagy-related and lysosomal proteins—including V0-ATPase and cathepsin D—have been documented in DS brain and are associated with impaired LC3-II formation [19]. Chronic inflammatory signaling also contributes to autophagy modulation [6]. Collectively, autophagy impairment in DS is early, persistent, and multifactorial, contributing to accumulation of misfolded proteins and dysfunctional organelles.

6. Interplay Between UPS and Autophagy in Down Syndrome

Maintenance of proteostasis depends on coordinated activity of UPS and autophagy within an integrated degradation network. In DS brain, dysfunction of these systems appears synergistic rather than compensatory. Reduced proteasomal activity and oxidative modifications of proteasome components [111] coexist with chronic mTOR hyperactivation and lysosomal impairment suppressing autophagic flux [89,91] . Proteomic analyses further reveal widespread alterations in proteins involved in degradation and redox regulation [40]. Ubiquitin signaling constitutes a central convergence point. When proteasomal degradation is insufficient, ubiquitinated substrates are redirected toward selective autophagy via adaptor proteins such as p62/SQSTM1 [127]. However, in DS—where autophagy is simultaneously compromised—this compensatory mechanism becomes ineffective, leading to accumulation of ubiquitinated aggregates [128]. Redox imbalance further amplifies this collapse. Oxidative stress directly damages proteasome subunits and autophagy machinery [129], while defective mitophagy sustains mitochondrial ROS production [40]. Thus, oxidative stress acts both upstream and downstream of proteostasis dysfunction, reinforcing a feed-forward cycle of degradation failure. Within this integrated framework, coordinated impairment of UPS and autophagy emerges as a central driver of proteostasis exhaustion and accelerated Alzheimer-like neurodegeneration in Down syndrome, providing a strong mechanistic rationale for therapeutic strategies aimed at restoring global degradative capacity (**Figure 1).**

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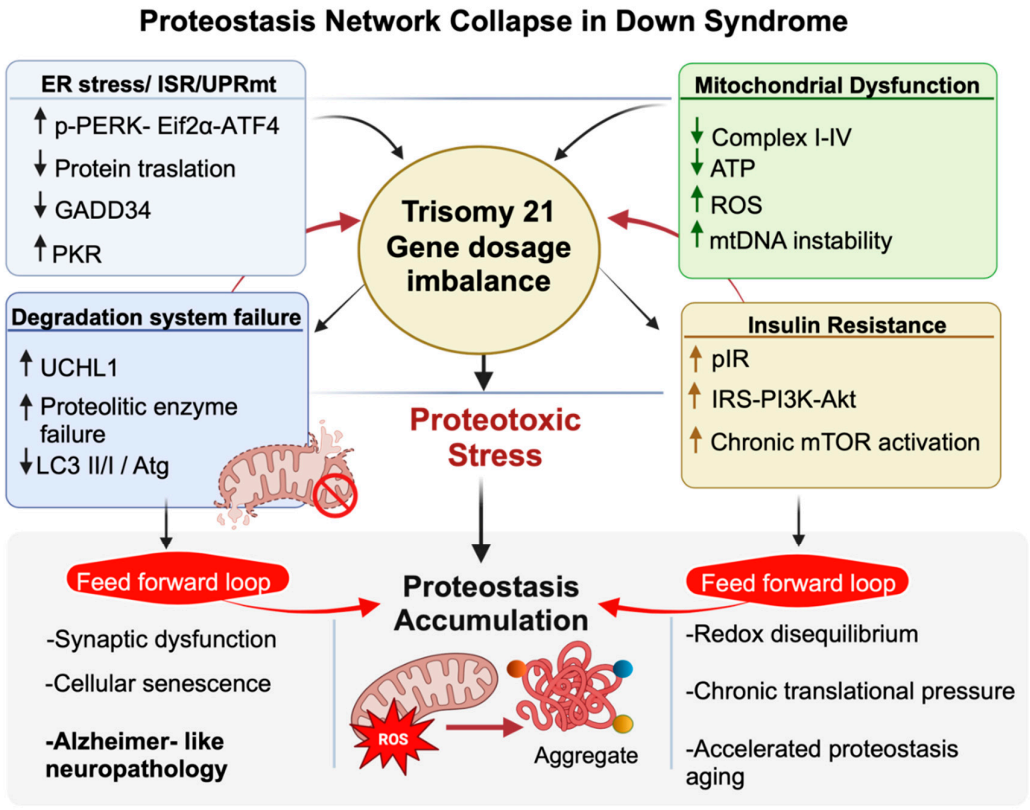

Figure 1. Schematic representation of proteostasis network collapse in Down syndrome. Trisomy 21–driven gene dosage imbalance perturbs multiple proteostasis modules, including endoplasmic reticulum (ER) stress and integrated stress responses (ISR/UPRmt), with increased PERK–eIF2*α*–ATF4 signaling, reduced global protein translation. Concomitantly, degradation pathways are impaired, as indicated by altered UCHL1 levels, defective proteolytic enzyme activity, and dysregulated autophagy (LC3 II/I and Atg proteins), leading to inefficient clearance of damaged proteins. Mitochondrial dysfunction, characterized by reduced respiratory chain complex I–IV activity, decreased ATP production, increased reactive oxygen species (ROS), and mitochondrial DNA instability, further exacerbates cellular stress. Insulin resistance, with increased IR phosphorylation, impaired IRS–PI3K–Akt signaling, and chronic mTOR activation, contributes to chronic translational pressure and impaired proteostasis maintenance. Together, these alterations converge in proteotoxic stress and progressive accumulation of misfolded/aggregated proteins, which in turn sustain "feed forward" loops that promote synaptic dysfunction, cellular senescence, redox disequilibrium, accelerated proteostasis aging, and Alzheimer like neuropathology in Down syndrome.

7. Therapeutic Implications

The central involvement of degradation systems in DS proteostasis imbalance identifies multiple therapeutic entry points. Rather than targeting individual pathogenic proteins, increasing evidence supports strategies aimed at restoring global proteostasis capacity and redox resilience through modulation of interconnected stress-response and clearance pathways. Several studies demonstrated the ability of different compounds to target components of the proteostasis network in both preclinical and clinical studies (Table1).

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Table 1. List of all the compounds used in DS neuropathology in clinical and pre-clinical studies.

Compound	Target	study type	Dosage	Length of the treatment	Administration route	Model	Ref.	Outcomes
Unfolded Protein Response and Integrated stress Response inhibitors								
GSK2606414	PERK	Preclinical study	0.1 μg/μl	5 days (1 x day)	intranasal treatment	Ts2Cje	[26]	Restored protein synthesis; reduced OS
ISRIB	eiF2a	Preclinical study	2.5 mg/kg	7 days (once every 2 days)	i.p. injection	Ts65dn	[27]	Restored protein synthesis; improved long-term memory
PKRi	PKR	Preclinical study	0.1 mg/kg	6 days (1 x day)	i.p. injection	Ts65dn	[135–137]	Rescued long-term memory and synaptic plasticity
Fluoxetine	PKR (indirect)	Preclinical study	Not specified	Early postnatal	Systemic	Ts65dn	[135–137]	Rescued long-term memory; neurogenesis
UPS modulators								
USP16	USP16	Preclinical study	Not specified	Not specified	In vitro	Ts65Dn DS stem cells	[143]	Rescued proliferation defects
Rapamycin	mTOR	Preclinical study	1ug	90 days (1x day,3x week)	Intranasal	Ts65Dn	[93,125]	Reduced Lys63-linked polyubiquitinated proteins
Autophagy modulators								
AOAA	CBS/H_2S pathway	Preclinical study	1 mg/kg/day	14 days, daily administration	intraperitoneally	Dp(17)3Yey/+	[132]	Improved cognition; restored autophagy
Rapamycin	mTOR	Preclinical study	1ug	90 days (1x day,3x week)	Intranasal	Ts65Dn	[93,125]	Reduced APP/tau pathology; rescued hippocampal tasks
		Preclinical study	1mg/kg	3 consecutive days during gestation	i.p. injection to pregnant dams	Ts1Cje	[130]	Corrected synaptic plasticity;
		Preclinical study	10 mg/kg	5 days (1 x day)	i.p. injection	Ts1Cje	[133]	Restored spatial long-

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								term memory
AZD8055	mTORC1/2	Preclinical study	0,1 uM	2, 4 and 8h	In vitro	Human fibroblasts	[89]	Restored autophagy and mitophagy
Metformin	AMPK/mTOR	Preclinical study	0,5mM	72h	In vitro	Human T21 fibroblasts	[78,89]	Restored mitophagy and lysosomal clearance
Polydatin	Mitophagy; miR-155	Preclinical study	10uM	24-72h	In vitro	Human T21 fibroblasts	[85]	Mitochondrial bioenergetics and mitophagy
KYCCSRK peptide	BVR-A	Preclinical study	0,5mM	2 weeks	Intranasal	Ts2Cje	[134]	Restored insulin signaling and mitochondrial function
Thiamet G	O-GlcNAcylation	Preclinical study	25 ug	5 days (2×day)	Intranasal	Ts2Cje	[106]	Boosted autophagy induction
Antioxidants								
		Preclinical study	50 mg/Kg	5 months	diet supplementation	Ts65dn	[144]	Reduced OS; improved spatial working memory
		Preclinical study	0.1% w/w for Kg of diet	Pregnancy and pups	maternal supplementation	Ts65dn	[146]	Improved cognition; reduced lipid peroxidation
α-Tocopherol	ROS	Randomized, double-blind, placebo-controlled trial	900 IU+ ascorbic acid (200 mg) + α-lipoic acid (600 mg)	2 years (daily)	oral	DS and AD individuals	[145]	No cognition improvement
		Randomized, placebo-controlled clinical trial	1000 IU	over 3 years (twice daily)	oral	DS over 50 years	[168]	No cognitive improvement
		randomized	266 mg + α-lipoic	4-months (daily)	oral	DS children	[169]	Reduced OS at DNA level

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		controlled trial	acid (100 mg/day)					
		Clinical study	400 mg + Vitamin C (500 mg/day)	over 6 months (daily)	oral	DS children and teenagers	[170]	Reduced blood levels of lipid peroxidation
CoQ10	Mitochondrial ETC	Clinical study	4 mg/kg/day	6 or 20 months (daily)	Oral	Children DS	[149]	Inhibited DNA oxidative damage; inconsistent long-term effects
		Clinical study	4 mg/kg/day	4-year (daily)	Oral	Children DS	[148]	No reduced OS level level at RNA or DNA level
Apigenin	NF-κB; antioxidant	Preclinical study	2 μM (in vitro); 200-250 mg/kg/day (in vivo)	Prenatal+postnatal	Oral/systemic	T21 amniocytes; Ts1Cje	[157,158]	Reduced OS; improved spatial memory (sex-specific)
7,8-DHF	TrkB (BDNF mimetic)	Preclinical study	5 mg/kg	Postnatal treatment: for 12 days, Adult treatment for 40 days.	Subcutaneous administration	Ts65Dn	[157]	Restored mitochondrial bioenergetics; increased
Melatonin	ROS scavenger	Preclinical study	10 mg/kg/die	5 months	Oral	Ts65Dn	[161–163]	Improved spatial learning; reduced lipid peroxidation
			10 mg/kg/die	6 months	Oral	Ts65Dn	[161–163]	reduced OS and hippocampal senescence
Metformin	AMPK/NF-κB	Preclinical study	10,30,50 uM	48h	Systemic	Human T21 fibroblasts	[164,165]	Mitigated oxidative damage
Lithium	REST	Preclinical study	10mM	24h	In vitro	iPSC-derived neurons	[165]	Restored REST levels; reduced OS
CAPE	BACH1/NRF2	Preclinical study	10 μM	6h	In vitro	human DS lymphoblastoid (LCLs)	[37]	Promoted NRF2 activation

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VP961	BACH1 /NRF2	Preclinic al study	5 μM	6h	In vitro	human DS lymphobla stoid (LCLs)	[37]	Promoted NRF2 activation
GLP-1 (cleavage product)	GLP-1R; mitocho ndrial ROS	Preclinic al study	500ng/g	2-3 weeks	Ip injection	9 mo Ts65Dn	[166]	Decreased mitochondri al OS
EGCG	DYRK1 A; ROS	Preclinic al study	20 μM	72 h (changed every 24 hours)	cells treatment	Human DS cell cultures	[151]	Reduced OS and mitochondri al energy deficit
		Preclinic al study	2–3 mg/day	1 month	water supplementat ion	Ts65Dn/Tg Dyrk1A	[153]	Improved cognition
		Preclinic al study	225 mg/kg/da y	4 weeks	water supplementat ion	Ts65Dn	[155]	Restored excitatory/in hibitory (E/I) imbalance (GABA modulation)
		Preclinic al study	25 mg/Kg/da y	P3 to P15	subcutaneous injection	Ts65Dn	[171]	Restored neurogenesi s at P15; no cognitive improvemen t at P45
		Preclinic al study	30 mg/kg/ day	30 days	water supplementat ion	Ts65Dn	[152]	Rescued CA1 dendritic spine density, improved cognition
		Preclinic al study	50 mg/kg	T1 (21 days) T2 (mating until 90 days) T3(P60-P90)	diet supplementat ion	Dp(16)1Ye y	[154]	Rescued GAD67; restored VGAT1/VG LUT1 balance; improved novel object recognition memory
		phase I randomi zed controlle d clinical trials	9 mg/kg/da y	6 and 12 months	diet supplementat ion	Young adults with DS	[153] [172]	Reduced plasma homocystein e; rescued cognitive performance s

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7.1. Autophagy Modulation

Autophagy has emerged as a pivotal node linking mitochondrial dysfunction, proteotoxic stress, and neurodegeneration in DS. Several pharmacological interventions targeting the Akt/mTOR axis have demonstrated the ability to restore impaired autophagic flux and improve protein clearance [89,130] [131]. In DS models, inhibition of mTOR signaling consistently reactivates autophagy and ameliorates downstream neuropathology. Aminooxyacetic acid (AOAA), a cystathionine β-synthase inhibitor, corrects CBS/H_2S pathway overactivity in Dp(17)3Yey/+ mice, improving cognition and synaptic ATP production while attenuating ER stress, gliosis, and autophagic alterations [132]. Rapamycin represents the most extensively studied autophagy modulator in DS. Intranasal rapamycin administration in Ts65Dn mice normalizes hippocampal and cortical mTOR signaling, restores autophagic flux (increased LC3-II and autophagy-related proteins), improves insulin signaling, reduces APP processing and tau hyperphosphorylation, and rescues hippocampal-dependent memory [93,125]. Prenatal rapamycin treatment in Ts1Cje mice corrects synaptic plasticity abnormalities and spine morphology alterations, highlighting developmental sensitivity of mTOR-dependent mechanisms [130]. Similarly, short-term intraperitoneal rapamycin restores spatial long-term memory in Ts1Cje mice, indirectly implicating autophagy induction in cognitive rescue [133]. Second-generation mTOR inhibitors such as AZD8055 restore macroautophagy and PINK1/PARKIN-dependent mitophagy in DS fibroblasts, rescuing mitochondrial clearance [89,130] . Metformin likewise improves mitophagy and lysosomal function in trisomic cell [78,89], reinforcing the therapeutic tractability of nutrient-sensing pathways. Additional interventions converge on mitochondrial-autophagy crosstalk. Polydatin reactivates mitochondrial bioenergetics and mitophagy while preventing premature senescence via modulation of microRNA-155 in T21 fibroblasts [85]. Intranasal Thiamet G restores O-GlcNAcylation, enhances autophagy induction, and rescues mitochondrial function in DS models [106]. The KYCCSRK peptide, derived from Biliverdin Reductase-A, restores brain insulin signaling, reduces oxidative damage, and limits amyloidogenic processing, indirectly reinforcing proteostasis capacity [134]. Collectively, these findings indicate that carefully titrated activation of autophagy and mitophagy represents a promising strategy to counteract degradation insufficiency in DS.

7.2. ISR and UPR Modulation

Chronic activation of the integrated stress response (ISR), characterized by persistent eIF2α phosphorylation, contributes to translational repression and impaired synaptic plasticity in DS [27]. Pharmacological inhibition of PKR using small-molecule inhibitors (PKRi) or early postnatal fluoxetine administration restores hippocampal neurogenesis and long-term memory in Ts65Dn mice [135–137]. ISRIB, a potent eIF2B activator, rescues de novo protein synthesis and improves memory performance in DS models [27], directly demonstrating the pathological relevance of sustained ISR activation. Targeting the PERK arm of the UPR has also shown therapeutic promise. Intranasal administration of the PERK inhibitor GSK2606414 in Ts2Cje mice reduces chronic PERK signaling, restores translational balance, and reactivates Nrf2-dependent antioxidant responses by correcting the Nrf2/Bach1 imbalance [26]. Beyond ER stress, early mitochondrial stress responses (UPRmt) have emerged as additional targets. Altered ATF5/GRP75 signaling in Ts2Cje frontal cortex contributes to early oxidative distress [138], identifying mitochondrial proteostasis pathways as potential intervention nodes.

7.3. Ubiquitin–Proteasome System (UPS) Targeting.

Given early proteasomal impairment in DS brain, modulation of the UPS represents an additional therapeutic frontier. Trisomy 21 includes genes encoding deubiquitinases such as USP16 and USP25. Inhibition of USP16 rescues stem cell proliferation defects in DS models [139–141], while USP25 overexpression has been implicated in impaired neurogenesis and cognitive deficits, nominating it as a potential pharmaceutical target [142]. Importantly, crosstalk between UPS and

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autophagy suggests that enhancing autophagic clearance may relieve proteasomal burden. Intranasal rapamycin reduces accumulation of Lys63-linked polyubiquitinated proteins in Ts65Dn mice without affecting Lys48 linkages, indicating selective rebalancing of degradation pathways [143]. These findings support a systems-level approach in which coordinated restoration of UPS and autophagy may prove more effective than isolated pathway targeting.

7.4. Antioxidant and Redox-Modulating Strategies

Given the central role of oxidative stress in destabilizing proteostasis, antioxidant-based approaches have long been explored in DS. Preclinical studies demonstrated beneficial effects of α-tocopherol supplementation in Ts65Dn mice, including reduced lipid peroxidation, attenuation of cholinergic degeneration, and improved spatial memory [144–146]. However, large randomized clinical trials in adults with DS and AD failed to show significant cognitive benefit despite multi-year antioxidant administration [147]. Coenzyme Q10 improved oxidative DNA damage markers in children with DS, although long-term efficacy remained inconsistent [148–150]. Among polyphenols, epigallocatechin gallate (EGCG) has received the most attention. In Ts65Dn mice, EGCG restores neurogenesis, rebalances excitatory/inhibitory transmission, and improves learning [144,151–155]. Clinical trials in young adults demonstrated modest improvements in memory and executive function after 12 months of treatment [137,152–155]. Beyond DYRK1A inhibition, EGCG also modulates epigenetic regulators such as DNMT1 and ADAR1 and influences homocysteine metabolism and MMP/TIMP balance, potentially affecting amyloid dynamics [156]. Other flavonoids, including apigenin and 7,8-dihydroxyflavone (7,8-DHF), improve neurodevelopmental outcomes and mitochondrial bioenergetics in DS models [157–160] . Melatonin reduces oxidative damage and improves spatial learning in Ts65Dn mice [161–163]. Metformin modulates AMPK/NF-κB signaling and mitigates oxidative stress [164], while lithium restores REST levels and reduces oxidative damage in DS-derived neurons [165]. CAPE and its analogue VP961 restore the BACH1/NRF2 axis, promoting ARE-driven cytoprotective gene expression [37]. GLP-1 receptor agonists have also shown neuroprotective and antioxidant effects. The GLP-1 cleavage product improves hippocampal LTP and cognition in Ts65Dn mice [166], and other GLP-1RAs activate the Nrf2/HO-1 axis in neurodegenerative models [167], providing a rationale for further exploration in DS.

Taken together, therapeutic evidence in DS increasingly supports a shift from single-target interventions toward strategies aimed at restoring integrated proteostasis networks. Modulation of mTOR signaling, ISR/UPR pathways, mitochondrial quality control, UPS components, and redox balance converges on a shared objective: re-establishing cellular buffering capacity against chronic proteotoxic and oxidative stress **(Figure 2).** Because proteostasis failure in DS is developmentally established and progressively amplified across the lifespan, timing of intervention may be critical. Early modulation of stress-response and degradative pathways could potentially delay or attenuate the trajectory toward Alzheimer-like neurodegeneration.

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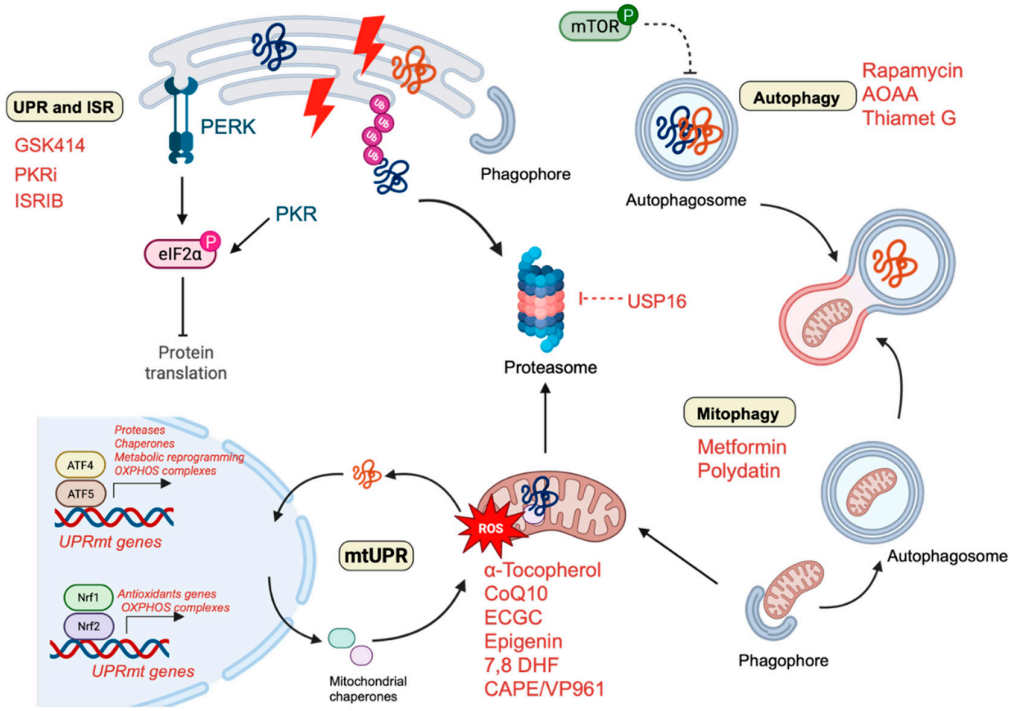

Figure 2. Major components of the proteostasis network altered in Down syndrome and their pharmacological modulators. In the autophagy panel (right), inhibition of mTOR by rapamycin, AOAA, and Thiamet G restores autophagic flux, promoting autophagosome formation and maturation into autolysosomes. Metformin and polydatin enhance mitophagy, facilitating the selective removal of damaged mitochondria. In the ER-stress and ISR/UPR panel (upper left), activation of PERK and PKR leads to eIF2α phosphorylation and suppression of global protein translation; GSK2606414, PKRi, and ISRIB are shown as pharmacological modulators that attenuate stress signaling and help rebalance protein synthesis. The central proteasome panel highlights the role of the deubiquitinase USP16 in proteasomal dysfunction and the accumulation of ubiquitinated substrates, emphasizing the ubiquitin–proteasome system as a critical hub of proteostasis control. In the mitochondrial UPR and redox panel (lower left), mitochondrial stress and excessive reactive oxygen species (ROS) production are counteracted by α-tocopherol, CoQ10, EGCG, apigenin, 7,8-dihydroxyflavone, and CAPE/VP961, which support mitochondrial proteostasis and antioxidant responses. Together, these pathways illustrate a network-based therapeutic strategy aimed at restoring autophagy and mitophagy, ISR/UPR signaling, ubiquitin–proteasome function, and redox homeostasis to improve cellular proteostasis in Down syndrome.

Concluding Remarks

DS should no longer be conceptualized solely as a gene-dosage disorder, but rather as a systems-level perturbation of proteostasis networks established from early development. Trisomy 21 imposes chronic translational pressure, redox disequilibrium, and degradation pathway insufficiency that progressively erode cellular resilience across the lifespan. Sustained ER stress with PERK-dominant signaling, incomplete mitochondrial stress adaptation, impaired ubiquitin–proteasome activity, and suppressed autophagic flux converge into a coordinated failure of proteostasis. Rather than isolated defects, these alterations form an interconnected vulnerability network in which mitochondrial dysfunction, oxidative stress, and degradation insufficiency reinforce one another in a feed-forward manner. Within this framework, insulin resistance and chronic mTOR hyperactivation function as metabolic switches that lock trisomic cells into maladaptive stress signaling states, limiting adaptive clearance responses and amplifying proteotoxic burden. Alzheimer-like neuropathology in DS thus emerges not simply as a consequence of APP triplication, but as the late manifestation of decades-long proteostasis exhaustion. This integrated perspective reframes DS as a human model of

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premature aging, providing unique insight into the mechanistic interface between redox imbalance, mitochondrial fragility, and neurodegeneration. Therapeutic strategies aimed at restoring global proteostasis capacity rather than targeting individual aggregates may offer broader and more durable benefits, particularly if implemented during early developmental or preclinical windows.

Future research should prioritize longitudinal systems-level approaches to identify biomarkers of proteostasis resilience and define optimal timing for intervention. In doing so, DS may serve not only as a target for precision therapeutic development, but also as a translational framework for understanding the broader biology of age-related neurodegeneration.

Data Availability Statement: No new data were created or analyzed in this study.

Conflicts of Interest: The authors declare that no conflict of interest exists.

Abbreviations

The following abbreviations are used in this manuscript:

AD – Alzheimer's disease
AMPK – AMP-activated protein kinase
ANT1 – Adenine nucleotide translocator 1
AOAA – Aminooxyacetic acid
APP – Amyloid precursor protein
ARE – Antioxidant response element
ATF4 – Activating transcription factor 4
ATF5 – Activating transcription factor 5
ATF6 – Activating transcription factor 6 (sensore UPR)
ATP – Adenosine triphosphate
Aβ (A-beta) – Amyloid-β / Amiloide-beta
BACH1 – BTB and CNC homology 1
BCL-2 – B-cell lymphoma 2
BIR – Brain insulin resistance
C99 – C-terminal 99-amino acid APP fragment
CAPE – Caffeic acid phenethyl ester
CBS – Cystathionine β-synthase
CHOP – C/EBP homologous protein
Chr21 – Chromosome 21
CNS – Central nervous system
CoQ10 – Coenzyme Q10
DNMT1 – DNA methyltransferase 1
DRP1 – Dynamin-related protein 1
DS – Down syndrome
DSAD – Down syndrome with Alzheimer's disease neuropathology
DYRK1A – Dual-specificity tyrosine-regulated kinase 1A
EGCG – Epigallocatechin gallate
eIF2α (eIF2-alpha) – Eukaryotic initiation factor 2 alpha
eIF2B – Eukaryotic initiation factor 2B
EOAD – Early-onset Alzheimer's disease
ER – Endoplasmic reticulum
ETC – Electron transport chain
ETS2 – ETS proto-oncogene 2
FUNDC1 – FUN14 domain containing 1
GADD34 – Growth arrest and DNA damage-inducible protein 34
GLP-1 – Glucagon-like peptide-1

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GLP-1RA – GLP-1 receptor agonist
GRP75 – 75 kDa glucose-regulated protein
GRP78/BiP – 78 kDa glucose-regulated protein / Binding immunoglobulin protein
H_2S – Hydrogen sulfide
HO-1 – Heme oxygenase 1
Hsa21 – Homo sapiens chromosome 21
HSP70 – Heat shock protein 70
iPSC – Induced pluripotent stem cell
IRE1 – Inositol-requiring enzyme 1
IRS – Insulin receptor substrate
ISR – Integrated stress response
LC3 – Microtubule-associated protein 1 light chain 3
LTP – Long-term potentiation
MFN2 – Mitofusin 2
miR-155-5p – microRNA-155-5p
MMP – Matrix metalloproteinase
MQC – Mitochondrial quality control
mtDNA – Mitochondrial DNA
mTOR – mammalian target of rapamycin
mTORC1 – mTOR complex 1
NF-κB (NF-kappa-B) – Nuclear factor kappa-light-chain-enhancer of activated B cells
NRF1 – Nuclear respiratory factor 1
NRIP1 – Nuclear receptor interacting protein 1
Nrf2 – Nuclear factor erythroid 2-related factor 2
OPA1 – Optic atrophy 1
OXPHOS – Oxidative phosphorylation
p16INK4a – Cyclin-dependent kinase inhibitor 2A
p21 – Cyclin-dependent kinase inhibitor 1
p53 – Tumor protein p53
p62/SQSTM1 – Sequestosome 1
PBMC – Peripheral blood mononuclear cell
PERK – Protein kinase R-like ER kinase
PET – Positron emission tomography
PGC-1α (PGC-1-alpha) – Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PI3K – Phosphoinositide 3-kinase
PINK1 – PTEN-induced kinase 1
PKR – Protein kinase R (EIF2AK2)
PKRi – PKR inhibitor
RCAN1 – Regulator of calcineurin 1
REST – RE1-silencing transcription factor
RNS – Reactive nitrogen species
ROS – Reactive oxygen species
SASP – Senescence-associated secretory phenotype
SIRT3 – Sirtuin 3
SLC25A4 – Solute carrier family 25 member 4 (ANT1)
SOD1 – Superoxide dismutase 1 (Cu/Zn-SOD)
TFAM – Mitochondrial transcription factor A
TIMP – Tissue inhibitor of metalloproteinases
UCH-L1 – Ubiquitin carboxyl-terminal hydrolase L1
UDP-GlcNAc – Uridine diphosphate N-acetylglucosamine
ULK1 – Unc-51-like kinase 1

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UPR – Unfolded protein response
UPRmt – Mitochondrial unfolded protein response
UPS – Ubiquitin–proteasome system
USP16 – Ubiquitin-specific peptidase 16
USP25 – Ubiquitin-specific peptidase 25
V0-ATPase – Vacuolar-type H^+-ATPase

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