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REVIEW

Physiological functions and clinical implications of the N-end rule pathway

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Abstract **The N-end rule pathway is a unique branch of the ubiquitin-proteasome system in which the determination of a protein's half-life is dependent on its N-terminal residue. The N-terminal residue serves as the degradation signal of a protein and thus called N-degron. N-degron can be recognized and modifed by several steps of post-translational modifications, such as oxidation, deamination, arginylation or acetylation, it then polyubiquitinated by the N-recognin for degradation. The molecular basis of the N-end rule pathway has been elucidated and its physiological functions have been revealed in the past 30 years. This pathway is involved in several biological aspects, including transcription, differentiation, chromosomal segregation, genome stability, apoptosis, mitochondrial quality control, cardiovascular development, neurogenesis, carcinogenesis, and spermatogenesis. Disturbance of this pathway often causes the failure of these processes, resulting in some human diseases. This review summarized the physiological functions of the N-end rule pathway, introduced the related biological processes and diseases, with an emphasis on the inner link between this pathway and certain symptoms.**

Keywords N-end rule pathway; Ate1; cardiovascular development; neurogenesis; spermatogenesis; neurodegenerative disorders; Johanson–Blizzard syndrome

Introduction

Since ubiquitin was discovered in 1977 [1], the ubiquitin-proteasome system (UPS) has emerged as an important pathway for protein degradation. This system regulates numerous irreversible proteolytic processes and maintains cellular homeostasis [2,3]. UPS dysfunction often causes serious human diseases, including chronic neurodegenerative diseases, such as Lewy body dementia and Parkinson's disease [4,5], spongiform degenerative disorders [6], skeletal muscle atrophy [7], cardiac dysfunction [8], chronic kidney disease [9], and colorectal cancer [10]. Recently, UPS has also been considered as an important drug target for these diseases and has provided several possibilities for drug discovery.

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UPS comprises a special branch called the N-end rule pathway that a protein's *in vivo* half-life is determined by its N-terminal residue [11,12]. This pathway was discovered in 1986, when Varshavsky and his colleagues found that some engineered products generated from enzyme-cleaved ubiquitin fusion proteins are unstable in *Saccharomyces cerevisiae* [13]. Since then, the physiological substrates of the N-end rule pathway have been expanded continuously, and it participates in multiple physiological processes and regulates various crucial biological processes [12,14]. In addition, various evidences and reports show that defects in the N-end rule pathway might lead to human diseases [15–18].

In this review article, we introduce the biochemical machinery of the mammalian N-end rule pathway, and summarize its recently identified substrates together with their related biological functions in mammals. Moreover, we illustrate the relationship between the dysfunction of the N-end rule pathway and certain human diseases and discuss some potential therapeutic strategies.

Overview of the N-end rule pathway

The N-end rule pathway relates a protein's degradation determination to its N-terminal residue. This connection is realized by the three main components of the N-end rule pathway: N-degron, which is a target that contains destabilizing N-terminal residues; N-recognin, which is an E3 ubiquitin ligase that recognizes and polyubquitinates N-degrons to facilitate their degradation by the third component, proteasome. Various enzymes, including mammalian deaminase NTAN1, NTAQ1, arginyl-tRNA transferase ATE1 and N-terminal acetyltransferase [11,12,19–23], catalyze post-translational modifications also function as essential parts of the N-end rule pathway.

A functional N-degron is typically composed of a destabilizing N-terminal residue, an internal Lys residue, where a polyubiquitin chain is formed, and an unstructured N-terminal extension. The unique N-terminal destabilizing residue determines the degradation signal [24–26]. In eukaryotes, the N-end rule pathway consists of two branches: the Ac/N-end rule pathway and the Arg/N-end rule pathway. The Ac/N-end rule pathway recognizes proteins through their N^{α}-terminally acetylated (Nt-acetylated) residues. The Nt-acetylation is catalyzed by Nt-acetylases (NAT) and this irreversible process typically occurs co-translationally either at the retained N-terminal Met or at a newly exposed N-terminal residue, such as Ala, Val, Ser, Thr, or Cys after the N-terminal Met is constitutively removed by Met aminopeptidases (MetAPs), creating an Ac/N-degron [22,27–29]. Although the Ac/N-end rule pathway was first discovered in *Saccharomyces cerevisiae*, the degradation of substrates containing the destabilizing residue Met followed by Arg, Gln, or Leu is also dependent on the Ac/N-end rule pathway in mammals, involving a functional N-recognin Teb4 (Fig. 1A) [30]. In the Arg/N-end rule pathway, Asn, Gln, and Cys are tertiary destabilizing residues. Asn and Gln can be deamidated into the secondary destabilizing residues Asp and Glu by the N-terminal deamidases NTAN1 and NTAQ1 [31–34] and Cys can be oxidized to Cys-sulphinic acid ($CysO_2(H)$) or Cys-sulphonic acid ($CysO_3(H)$), both of them can serve as secondary destabilizing residues [35–37]. The N-terminal Asp, Glu, and oxidized Cys (marked as Cys*) are conjugated with Arg by ATE1, which creates the primary destabilizing residue Arg at the N terminus of an otherwise stable protein [38–40]. Arg, Lys, and His are three positively charged amino acids classified as Type I N-degrons. Five additional bulky hydrophobic amino acid residues, Phe, Leu, Trp, Ile, and Tyr along with Met followed by hydrophobic amino acid residues (marked as MΦ), are classified as Type II N-degrons [11]. The N-recognins in mammalian cells include UBR1, UBR2, UBR4, and UBR5. UBR1, 2, and 4 can bind to Types I and II degrons, whereas UBR5 shows a preference for Type I degrons [41]. Once activated by E1 activating enzymes and transferred to E2 enzymes, such as HR6A or HR6B, ubiquitin is conjugated to substrates by N-recognins to promote protein degradation (Fig. 1B).

Generation of N-degrons in N-end rule pathway

N-degrons are produced through co-translational or post-translational modification. In many cases, N-degrons are also generated through the cleavage of certain proteins by intracellular nonprocessive proteases, such as MetAPs, caspases, calpains, and separases, which are often followed by various modifications [42,43]. Most proteins are co-translationally and irreversibly Nt-acetylated by ribosome-associated Nt-acetylases to generate potential substrates of the Ac/N-end rule pathway [44,45]. The first substrate of the Ac/N-end rule pathway identified in mammals is regulator of G-protein signaling 2 (RGS2), there is a Gln followling with the initiator Met in RGS2, and it can be directly Nt-acetylated to create a representative substrate [30].

Met is cleaved by MetAPs when the following residue is Val, Gly, Pro, Ala, Ser, Thr, or Cys, which contains a small side chain, thus creating N-terminal residues that can enter either the Arg/N-end rule pathway or the acetylation-based pathway depending on their identity [12,35,37,46–49]. This class of substrates include a set of RGS proteins, such as RGS4, RGS5, and RGS16, whose cleavage by MetAPs yields an exposed Cys, and it is a tertiary destabilizing residue of the Arg/N-end rule pathway. A primary degron usually is created through arginylation following oxidation [35,37].

A classical N-degron can also be created through cleavage by nonprocessive proteases, such as caspases, separases, and calpains. This endoproteolytic cleavage of a stable protein may form a C-terminal fragment possessing a primary destabilizing residue, a known substrate in this class contains *S. cerevisiae* Scc1 [50], a subunit of cohesin complex, whose cleavage by separase is necessary to sister chromatids segregation during anaphase [51,52]. It also comprises substrates containing second/tertiary destabilizing residues, like separase-cleaved REC8, which is the meiotic counterpart of Scc1 [53], various apoptosis proteins cleaved by caspase or calpains, including breast cancer 1 (BRCA1), RIPK1 [54,55], and others listed in Table 1.

The internally embedded N-degrons upon translocation can be exposed through the cleavage of the signal sequence of a transported protein. Most proteins transported to the mitochondria contain pre-sequences that are removed by endopeptidases, such as mitochondrial processing peptidase (MPP) and presenilin-associated rhomboid-like (PARL) [56–60]. PARL can cleave the mitochondrial

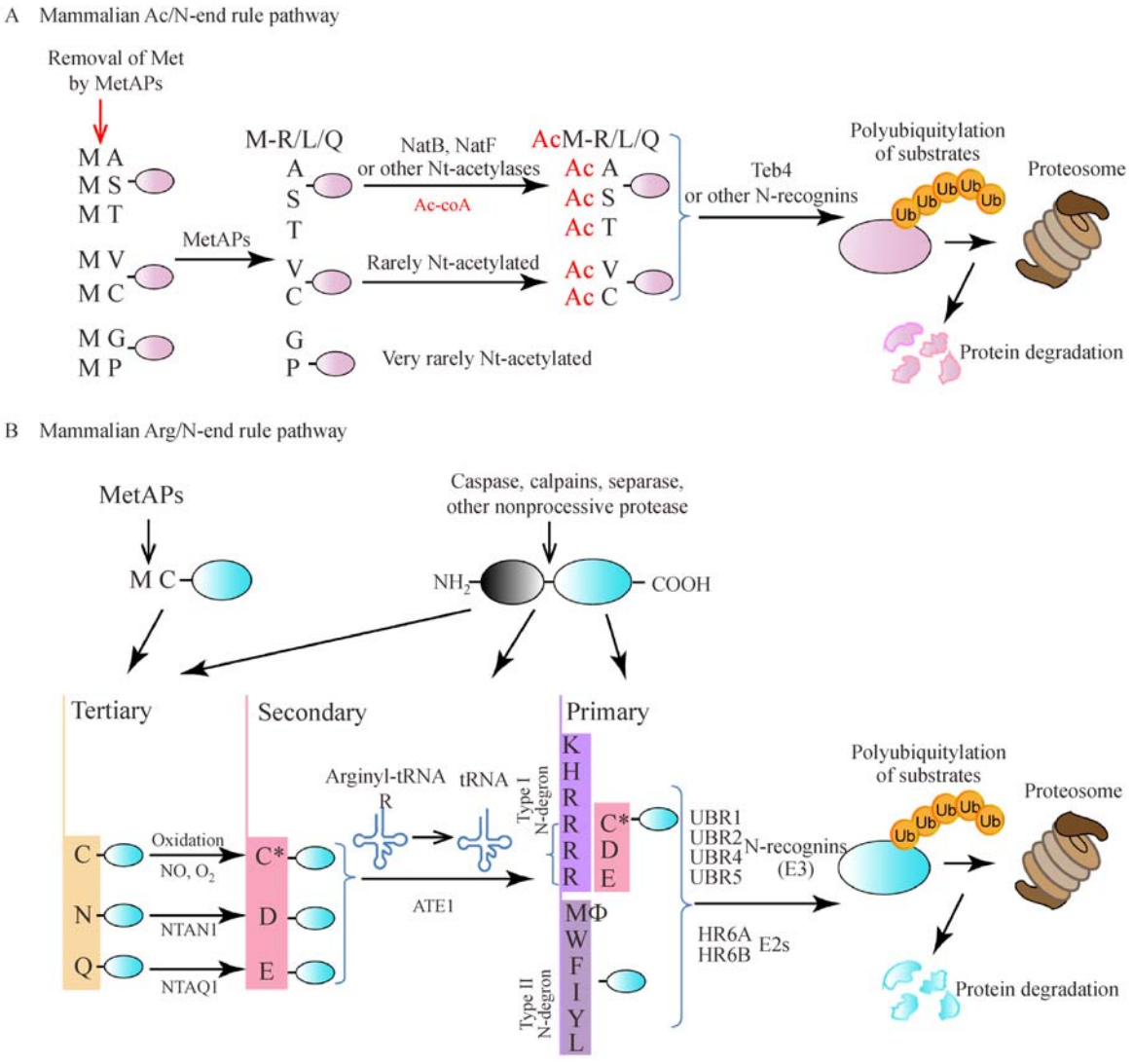

Fig. 1 Mammalian N-end rule pathway. (A) Mammalian Ac/N-end rule pathway. This pathway targets proteins through their N^{α}-terminally acetylated residues. The red arrow on the left indicates the co-translational removal of the initiator Met by Met-aminopeptidases (MetAPs). The N-terminal Met is retained when a residue at position 2 is larger than Val. (B) Mammalian Arg/N-end rule pathway. This pathway targets proteins for degradation through their specific unacetylated N-terminal residues. ATE1 is arginyl-tRNA-protein transferase. "Primary," "secondary," and "tertiary" refer to mechanistically distinct classes of destabilizing N-terminal residues. "Type I" and "Type II" refer to two sets of primary destabilizing N-terminal residues: basic and bulky hydrophobic, respectively.

membrane located E3 ubiquitin ligase PINK1, generating an exposed F-PINK1 fragment [17].

In summary, the generation of an N-degron involves intracellular nonprocessive proteolysis and various post-transcriptional modifications, including deamination, oxidation, and arginylation. N-degrons can be created through one or more steps of such processes. However, these modifications do not generate N-degrons in some instances, for example, β-actin can be acetylated at the N-terminal Met and induce the exposure of an internal Asp, which is arginylated by ATE1 [61,62]. This modification contributes to the proper assembly of actin filaments but does not facilitate their degradation [63].

Physiological function of the N-end rule pathway

The physiological functions of the N-end rule pathway are broad and have been extensively explored. Protein degradation regulated by the N-end rule pathway mediates several processes, including sensing of heme [64], NO, oxygen, and short peptides [35]; selective elimination of misfolded proteins [65,66]; the regulation of DNA repair,

Table 1 Representative substrates of the mammalian N-end rule pathway

Substrate	Species	N-degron	Modifications	References
RGS2	*Homo sapiens/Saccharomy cescerevisiae*	AcMQ-X	Acetylation	[30]
RGS4,5,16	*Mus musculus*	RC*-X	MetAPs cleavage, oxidation, arginylation	[35,37]
REC8	*Mus musculus*	E-X	Separase cleavage, arginylation	[53]
RIPK1	*Mus musculus*	C-X	Caspase cleavage, oxidation, arginylation	[54,55,116]
TRAF1	*Mus musculus*	C-X		
BRCA1	*Mus musculus*	D-X		
EPHA4	*Mus musculus*	D-X		
BIM_{EL}	*Mus musculus*	R-X		
MET	*Mus musculus*	T-X		
NEDD9	*Homo sapiens*	T-X		
LIMK1	*Homo sapiens*	L-X		
Lyn	*Homo sapiens*	L-X		
BID	*Homo sapiens*	R-X	Calpain cleavage, deamination, arginylation	[55,73]
BCL_{XL}	*Mus musculus*	D-X		
Bak	*Mus musculus*	E-X		
c-Fos	*Mus musculus*	R-X		
IκBα	*Mus musculus*	E-X		
Igfbp2	*Mus musculus*	R-X		
Capns1	*Mus musculus*	D-X		
Atp2b2	*Mus musculus*	R-X		
Capn1	*Homo sapiens*	L-X		
Ankrd2	*Mus musculus*	R-X		
Grm1	*Mus musculus*	T-X		
Ica512	*Mus musculus*	L-X		
PINK1	*Homo sapiens*	F-X	Transmembrane signal cleavage by PARL	[17]
APP	*Homo sapiens*	D-X	Secretase, calpain, caspase, or MMP3 cleavage; deamination; arginylation (see details in main text "Cancers")	[16]
Tau		E-X		
α-synuclein		Q-X		
TDP43		R208-TDP43		
		D219-TDP43		
		D247-TDP43		

Note: X represents C-terminal fragment of the corresponding proteins.

such as the degradation of Mgt1 [67,68]; chromosomes segregation, such as the degradation of a cohesin subunit [50,53]; signal transduction by transmembrane receptors, such as the degradation of G-protein regulators: RGS4, RGS5, and RGS16 [37]; control of peptide import, such as Ubr1-induced degradation of Cup9, which is the transcriptional repressor of a transmembrane peptide transporter [69,70]; regulation of leaf and shoot development, leaf senescence, and seed germination in plants [71,72]. The N-end rule pathway related functions in mammals also include anti-apoptosis through the degradation of a series of caspase- and calpain-cleaved pro-apoptotic fragments (Fig. 2 and Table 1) [54,55], mitochondrial quality control through the degradation of PINK1 [17], transcription regulation through the degradation of c-Fos and IκBα and cell differentiation and development through the degradation of insulin-like growth factor binding protein 2 (Igfbp2) [73]. This pathway is also closely related to fat metabolism [74] and organ development, such as brain, muscle, testes and pancreas [75]. The functional roles played by the N-end rule pathway in cardiovascular development, neurogenesis and spermatogenesis are well studied, and we will introduce this pathway in these three processes in detail. The major functions of the N-end rule pathway are summarized in Fig. 2.

N-end rule pathway in cardiovascular development

The cardiovascular system is well controlled by G protein-coupled receptor (GPCR) signaling cascades, whose imbalances can cause defects in cardiovascular development and function [76–78]. The activity of this pathway is modulated by regulators of G-protein signaling (RGS) that can act as GTPase-activating proteins for Gα, thus inhibiting G-protein signaling [37,79]. In the RGS family, several proteins, including RGS4, RGS5, and RGS16 in the Arg/N-end rule pathway [37,80], and RGS2 in the Ac/N-end rule pathway [30], have been defined as substrates of the N-end rule pathway. The degradation of these proteins is essential for the successful development and normal function maintenance of the cardiovascular system.

Mice that lack ATE1 R-transferase, an essential component of the Arg/N-end rule pathway that add Arg to the N-terminal Glu, Asp, or Cys* of proteins, have been

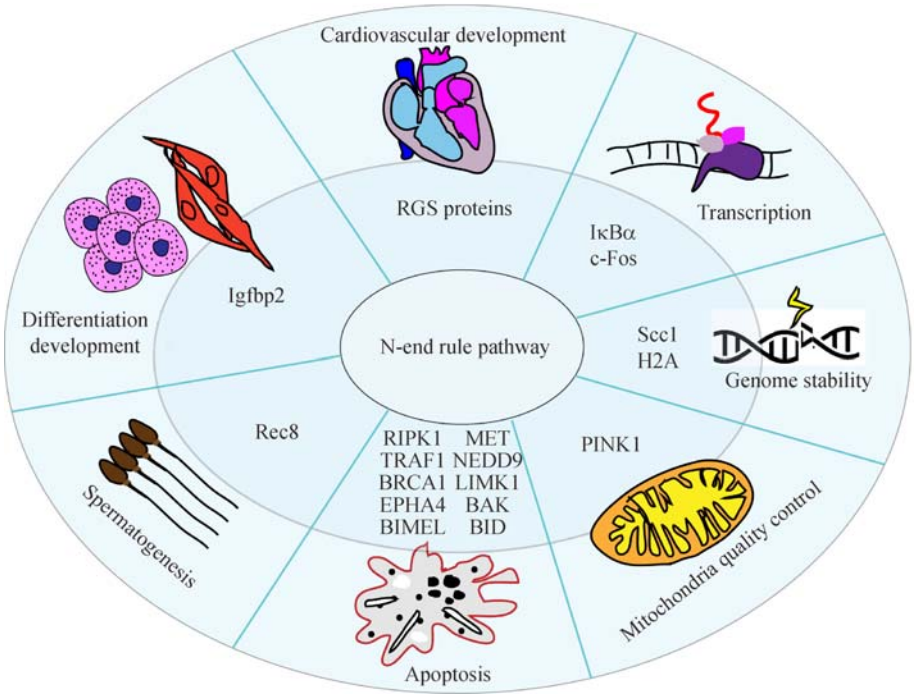

Fig. 2 Major biological function of the N-end rule pathway. The main biological functions of the N-end rule pathway include transcription through the degradation of IκBα and c-Fos, differentiation through the degradation of a growing factor Igfbp2, genome stability through the degradation of Scc1 and H2A, apoptosis through the degradation of a series of apoptotic proteins, mitochondrial quality control through the degradation of PINK, cardiovascular development through the degradation of RGS proteins, and spermatogenesis through the degradation of REC8.

reported as embryonical lethal. The mice died at E15.5–E16.5 with severe defects in carcinogenesis and exhibited thin-walled atrial septa, hypoplasic ventricular myocardium, and thin vessels, which are similar to the phenotype observed in *Ubr1* and *Ubr2* double-knockout mice [81]. Hemorrhage was observed consistently in the knockout mice and is likely the primary cause of lethality [36]. Further detailed studies have revealed that cardiac and vascular defects are independent of each other. Cardiomyocytes in *Ate1*-deficient embryos are impaired in proliferation accompanied by high RGS4 expression level. The misregulation of the Gα−PLC/PKC−MEK1−ERK1 axis of G-protein signaling is caused by the failure of RGS4 degradation and is accounted for major cardiac defects (Fig. 3) because Gα overexpression could rescue the ventricular septal defects and thin myocardium, but not the vascular defects [37].

RGS2 is another member of the RGS protein family that regulates stress responses, translation, circadian rhythms, and cardiovascular homeostasis [82–84]. Two mutants have been found in this protein in patients with hypertension, the second residue of wild-type MetGln-RGS2 (MQ-RGS2) is replaced by Leu or Arg [85]. These two mutants enhance the degradation of the RGS2 protein via Ac/N-end rule pathway, leading to higher activity of the MEK1-ERK1 signaling pathway and ultimately causing

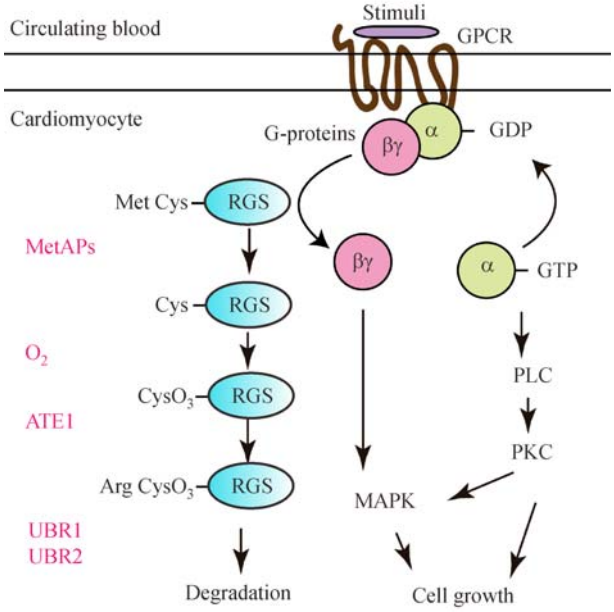

Fig. 3 Role of N-end rule pathway-mediated degradation of RGS proteins in cardiovascular development. G-protein coupled receptor (GPCR) transfers extracellular signals to the intracellular environment by dissolving heterotrimeric G-proteins and forming active Gα-GTP to stimulate downstream signal pathways. The RGS family acts as GTPase-activating proteins. As a result, the concentration of Gα-GTP in the cytosol decreases and downstream signaling is blocked.

hypertension [30].

In addition to the observed defects caused by abnormal degradation of RGS proteins, the myofibrils from the *Ate1*-deficient hearts also showed ultrastructural defects, including diffusing Z-bands and overall myofibril misalignment and disorganization, probably because a set of proteins that is directly involved in the contractility and/or the maintenance of the structural integrity of the myofibrils is arginylated [86]. This phenomenon suggests that arginylation also participates in cardiovascular development by regulating the structure of myofibrils in addition to mediating the degradation of specific proteins.

N-end rule pathway in neurogenesis

In addition to the cardiovascular defects in *Ubr1* and *Ubr2* double-knockout mice, severe neurogenesis defects were identified. In E10.5 $Ubr1^{-/-}Ubr2^{-/-}$ embryos, the neuroepithelium is thin, and obtains no increase in thickness afterward. And in E11.5 $Ubr1^{-/-}Ubr2^{-/-}$ embryos, neural tubes become strongly kinked. By E11.5, the morphological characteristics of the forebrain in the $Ubr1^{-/-}Ubr2^{-/-}$ embryos become grossly distorted, with curved, thin, and often disjointed neuroepithelial layers of varying thickness. Moreover, the distribution of neural progenitor cells in the ventricular zone (VZ) and differentiated cells in the differentiation zone (mantle) [87] is disrupted. These abnormalities may arise through the progression of developmental processes, such as cell proliferation, differentiation, and migration [81].

Further mechanistic investigations have revealed that the expression level of Cyclin D is reduced in $Ubr1^{-/-}Ubr2^{-/-}$ embryos, which may be relevant to the observed decrease in the number of neural precursor cells in $Ubr1^{-/-}Ubr2^{-/-}$ embryos. The Notch pathway in $Ubr1^{-/-}Ubr2^{-/-}$ embryos is suppressed due to the decreased expression of a transcription activator, Notch1. This phenomenon may partially account for the impaired neurogenesis. The mitogen-activated protein kinase (MAPK) pathway, which regulates cell proliferation, differentiation, and apoptosis, is also affected by *Ubr1* and *Ubr2* deletion, and the level of its activated form is substantially increased, causing exit from cell cycle and differentiation [81].

Although three related proteins are responsible for the neurogenesis defect in $Ubr1^{-/-}Ubr2^{-/-}$ embryos, the mechanisms by which UBR1 and UBR2 or the N-end rule pathway influences the expression or modification level of these proteins remain elusive. Further studies should be conducted to elucidate the relevant mechanisms.

N-end rule pathway in spermatogenesis

Meiosis is an essential sperm-production step in sexual reproduction. During meiosis, the number of chromosomes is reduced to half to create haploid spermatids. This reduction in chromosome number is achieved by two rounds of meiotic division with one round of DNA replication. In prophase I, many important events occur, including homologous chromosome pairing, recombination, and crossover with their partners to form chiasmata between non-sister chromatids in a bivalent chromosome [88,89]. The chiasmata are maintained by a cohesin complex to guarantee the precise segregation of homologous chromosomes [88,90,91]. It has been reported that the N-end rule pathway plays important roles in multiple aspects of male meiosis to ensure the successful progression of these processes.

UBR1 and UBR2 are important E3 recognins of the N-end rule pathway. In mice, the knockout of *Ubr1* did not influence the fertility of either males or females [92]. By contrast, $Ubr2^{-/-}$ male is infertile because of the failure of mature spermatozoa production [93], with a phenotype of pachytene stage arrest and masses of apoptotic spermatocytes. Further studies have demonstrated that spermatocytes in $Ubr2^{-/-}$ mouse testes remain normal during the DSB formation as RPA1 and RAD51 are successfully recruited to chromosomes. However, serious defects occur in DSB repair, synapsis, and crossover formation. Considering the function of UBR2 in ubiquitinating H2A and H2B, the above impairment may be related to a failure in bypassing the pachytene checkpoint [94]. The localization of UBR2 on chromosomes in prophase I is correlated with the "meiotic silencing of unsynapsed chromosomes" (MSUC) [95], a mechanism by which the chromatin linked to unsynapsed axes is silenced when homologous chromosomes undergo synapsis [96,97], and "meiotic sex chromosome inactivation" (MSCI) [98–100]. This phenomenon suggests that UBR2 may regulate spermatogenesis through transcription. The ubiquitination of H2A is involved in H2AX phosphorylation during MSCI [96,98,101]. Thus, UBR2 distributed on the chromatin may serve as a scaffold to promote HR6B/UbcH2-dependent ubiquitination of histone H2A and H2B through a special mechanism by which the E3 activity of UBR2 in histone ubiquitination is allosterically activated by dipeptides containing destabilizing N-terminal residues [95]. Meanwhile, the *Hr6b* knockout mice exhibit a spermiogenesis defect likely through a similar mechanism [102].

Along with prophase, our recent work demonstrated that the N-end rule pathway also participates in metaphase-to-anaphase transition in the first meiosis division [53]. In this stage, cohesins along the chromosome arms need to be removed to resolve the chiasmata for proper chromosome segregation [103,104]. The removal of cohesins is mediated by a protease called separase, and this protease can cleave the cohesin subunit REC8 in a site-specific manner to open the circular conformation to release the two entrapped chromosomes [105,106]. The cleavage of REC8 generates a C-terminal fragment bearing an N-

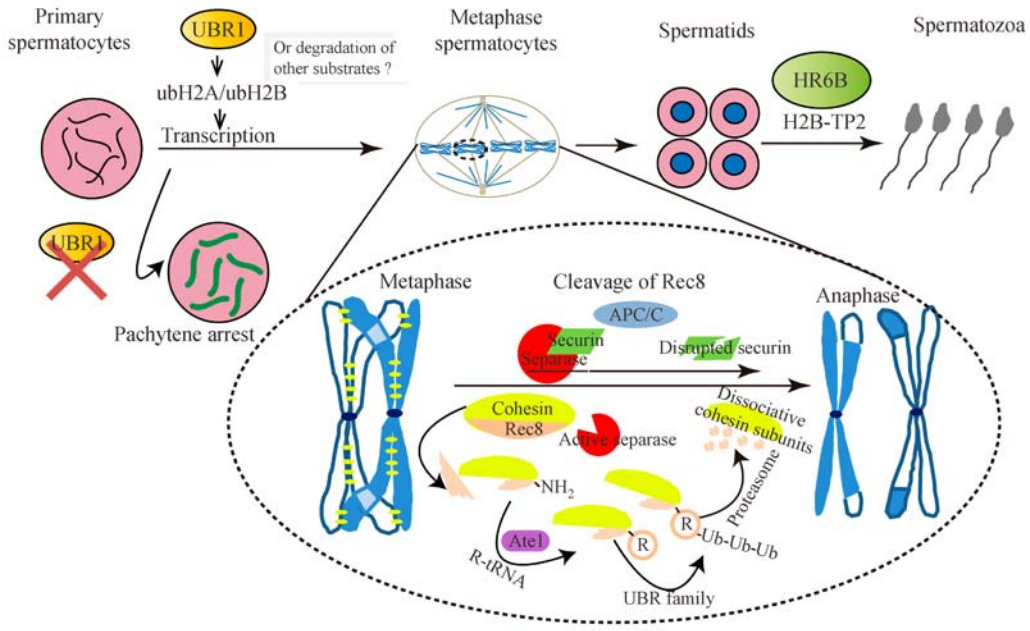

Fig. 4 Functional role of the N-end rule pathway in spermatogenesis. The N-end rule pathway participates in multiple stages of spermatogenesis. In prophase I, UBR1 facilitates the ubiquitination of H2A and H2B to maintain proper transcription and thus allow spermatocytes to bypass the pachytene checkpoint. In metaphase, the Ate1-mediated arginylation of the fragment of Rec8 cleaved by separase is required for its degradation. In spermiogenesis, histone replacement by protamine requires HR6B, which is the E2 of the N-end rule pathway.

terminal Glu, which is then arginylated by ATE1, thus creating an N-degron. Subsequently, the arginylated REC8 C-fragment is degraded via the N-end rule pathway. The abolition of arginylation during spermatogenesis through the conditional knockout of *Ate1* in primordial germ cells causes male infertility because of a remarkable reduction in the number of mature spermatozoa. Spermatocytes also undergo metaphase I arrest followed by apoptosis. Therefore, the Arg/N-end rule pathway is essential for proper chromosome segregation during meiosis by eliminating unnecessary proteins.

N-end rule pathway related human diseases

Johanson–Blizzard syndrome (JBS)

UBR1 (MIM #605981) mutations cause JBS (OMIM 243800) [75], which is an autosomal-recessive disorder first described by Ann Johanson and Robert Blizzard in 1971 [107]. The most common symptom of this syndrome is congenital exocrine pancreatic insufficiency, with other features involving facial malformations, such as nasal wing aplasia and scalp defects. Symptoms of JBS also include other facultative abnormalities, such as deafness, dental defects, hypothyroidism, urogenital and anorectal malformations. Moreover, mental retardation and cognitive impairment have also been reported [15,18,108,109]. The prevalence of this syndrome in Europe has been estimated at 1/250 000 [75].

The disease-associated locus is mapped to chromosome 15q14–21.1, where the *UBR1* gene is located. Versions of *UBR1* containing either truncated or single amino acids are associated with the disease. Truncated mutants are first discovered and described. They are caused by nonsense mutations, frame shift or splice-site mutations, presenting completely abolished functional protein product and hence are usually related to severe JBS symptoms. Milder JBS characteristics have occasionally been observed with missense mutations of *UBR1*.

Although more than 60 mutations of *UBR1* have been identified in patients with JBS, the molecular mechanism of the pathogenesis of JBS remains unclear. Moreover, there is no causal treatment available for patients affected by JBS, and symptoms have to be treated in accordance with general guidelines, such as enzyme supplementation for pancreatic insufficiency [108]. Considering the function of UBR1 in the N-end rule pathway and the absence or reduced activity of UBR1 in patients with JBS [75,108,110], it is reasonable to speculate that the disease may be caused by the aberrant degradation of some specific proteins in different tissue. Further research should

be performed to identify related substrates and to provide insights into the development of new treatment methods for JBS.

Cancers

Chronic myelogenous leukemia (CML) is a cancer of white blood cells. Imatinib is a commonly used drug in CML treatment by working as a tyrosine kinase inhibitor. This drug can induce cell cycle arrest or cell death, and it can control the proliferation of cancer cells. However, cancer cells exhibit resistance to this drug, which was observed in clinical practice [111,112]. The overexpression of some kinases, such as Lyn, is a common mechanism underlying drug resistance [113,114]. During the apoptosis of B cells, T cells, and CML cell line K562, Lyn is cleaved by caspase at Asp18, as a result, a leucine at the N-terminal becomes exposed [115–117] and a substrate of the N-end rule pathway is formed. This cleaved Lyn can function as an anti-apoptotic protein, and cells containing a stable form of a Lyn fragment exhibit an enhanced viability when they are treated with imatinib [112]. The N-end rule pathway works as a pro-apoptotic machinery that can directly enhance apoptosis and reduce cell proliferation by facilitating the degradation of an anti-apoptotic protein.

In addition, the N-end rule pathway also participates in other cancers. The loss or reduced levels of UBR1 expression cause errors in chromosome segregation, accelerating B cell lymphomagenesis [118] and cancer predisposition [119]. Deletion of UBR2 promotes cancer cachexia by accelerating muscle atrophy [120]. ATE1, which is another N-end rule pathway component, is correlated with metastases in human cancers, and ATE1-deficient embryonic fibroblasts exhibit tumorigenic properties [121]. Therefore, the N-end rule pathway plays an important role in various kinds of cancers, and it may be considered as a therapeutic target of disease treatments.

Neurodegenerative syndromes

Neurodegeneration refers to the progressive loss of neuronal structure or function, including neuronal death. Many neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, and Huntington's disease, occur because of neurodegenerative processes.

One common feature of neurodegenerative diseases is the accumulation of intracellular or extracellular neuronal protein aggregates [122,123]. Many aggregation-related proteins are short-lived substrates of the N-end rule pathway [16]. Aβ is an Alzheimer's disease-associated polypeptide containing 36 to 43 residues produced through the cleavage of amyloid precursor protein (APP) by secretases [124]. The 42-residue Asp-Aβ42 is a specific amyloid-derived species composed of an N-terminal Asp [125]. Similar to APP, Tau (isoform 2N), a microtubule-associated protein and a component of the intracellular aggregates, can be cleaved by calpains and caspases to produce a more aggregation-prone C-terminal fragment Glu3-Tau-2N-Gln124 [126,127]. α-Synuclein is a membrane-associated neuronal protein that functions in vesicular trafficking [128], and it forms large and toxic aggregates called Lewy bodies with a metalloproteinase 3 (MMP3)-cleaved Gln79-synuclein fragment [129–131]. TDP43 is an RNA/DNA binding protein and component of intracellular aggregates associated with TDP43 proteinopathies, including ALS [132]. Specific C-terminal TDP43 fragments, such as Arg208-TDP43, Asp219-TDP43, and Asp247-TDP43, are identified as predominant components of aggregates isolated from FTLD-TDP human brains. These fragments are more prone to aggregation than full-length TDP43 [133–135]. The natural fragments of these proteins comprise N-terminal-destabilizing residues that either can be recognized by the ubiquitin ligase E3 of the N-end rule pathway or can contain secondary or tertiary destabilizing residues, such as Asp or Gln, which can be modified through deamination and arginylation [11], permitting these fragments to enter the N-end rule-dependent degradation pathway [16].

With respect to the other aspect, mitochondrial dysfunction is a prominent characteristic of idiopathic Parkinson's disease and ALS [136]. Defects in mitochondria had previously been proposed to contribute to the occurrence of common neurodegenerative disorders because of increased neuronal cell death [137]. Therefore, mitochondrial dysfunction may be intrinsically related to neurodegenerative disorders. The N-end rule pathway also participates in the mitochondrial quality control by targeting the mitochondrial quality regulator PTEN-induced putative kinase 1 (PINK1) for degradation [17]. In addition to aggregation proteins, the N-end rule pathway is related to neurodegenerative disorders through mitochondria. PINK1 is a mitochondrial serine/threonine kinase that can be detected on the outer membrane of depolarized mitochondria [138,139]. This kinase recruits the E3 ubiquitin ligase Parkinson protein 2 (PARKIN) to induce their elimination through autophagy [138,140,141]. For a healthy mitochondria located PINK1, it is released to the cytosol after it is sequentially processed by a protease named matrix processing peptidase (MPP) in the matrix and presenilin-associated rhomboid-like (PARL) on the inner mitochondrial membrane [58–60]. The PARL-processed PINK1 contains an N-terminal Phe, which is a destabilizing signal that can be recognized by UBR1, UBR2, and UBR4 and sequentially degraded via the N-end rule pathway [17]. By eliminating PINK1 on healthy mitochondria, the N-end rule pathway facilities the efficient removal of dysfunctional mitochondria and thus may prevent the occurrence of potential neurodegenerative disorders to some extent.

Concluding remarks and future clinical prospects

After 30 years of investigations, numerous substrates of the N-end rule pathway have been identified, indicating that the N-end rule pathway is implicated in multiple biological processes and is necessary to maintain homeostasis in organisms. Moreover, additional substrates is still in discovery, suggesting that this pathway may also participate in other biological processes. Further studies should be conducted to obtain full knowledge of the regulatory mechanism of this pathway in various biological processes.

The dysfunction of the N-end rule pathway is related to diseases, such as JBS and neurodegenerative disorders. This pathway and its components are potential targets of new clinical therapeutics for these diseases. For example, UBR1 mutants can serve as a test subject for the first-trimester prenatal diagnosis in JBS-affected families. In addition, UBR1-targeted gene or stem cell therapy might provide an opportunity for JBS patients. Eliminating or reducing some accumulated N-end rule pathway substrates might help either treat related diseases or alleviate their symptoms. With respect to neurodegenerative disorders, making the aggregates suitable for degradation by the N-end rule pathway or enhancing the activity of some components to promote their elimination may be an efficient treatment method.

Considering the important roles of this pathway during development, deletion of most of its components except UBR1 could be lethal to humans. However, ATE1 has been found to be involved in a balanced translocation with the *SLC12A1* gene of a boy with a non-syndromic hearing loss. Moreover, the symptoms are very similar to those of JBS, indicating that these two types of mutants can affect physiological processes via a similar mechanism. Although the N-end rule pathway has yet to be explored in human cardiopathy or infertility, some N-end rule pathway mutants in human cardiac or germ cells may exist. These mutants unlikely affect the development of other organs, but they can cause defects in heart or sperms and thus induce diseases. Reciprocally, male infertility or cardiopathy may be caused by mutants in the N-end rule pathway. Thus, the N-end rule pathway components can be considered potential targets of therapeutic strategies against these diseases. Although dipeptides containing N-terminal degrons and dipeptide-mimetic molecules inhibit UBR1 and UBR2 activity [64,142], the N-end rule pathway-specific small molecular inhibitors are still urgently needed to be discovered to expand and diversify the range of therapies for affected patients.

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Compliance with ethics guidelines

Yujiao Liu, Chao Liu, Wen Dong, and Wei Li declare that they have no financial conflicts of interest. This manuscript is a review article and does not involve a research protocol requiring approval by a relevant institutional review board or ethics committee.

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