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NUBOPATHIES: NUB1-RELATED NEURODEGENERATIVE DISEASES

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Abstract NEDD8 ultimate buster-1 (NUB1) is a potent down-regulator of the ubiquitin-like protein NEDD8, because it directly interacts with NEDD8 and targets it and its conjugates to the 26S proteasome for proteolytic degradation. Recently, we found that NUB1 physically interacts with synphilin-1 through its NEDD8-binding site, implying that NUB1 also targets synphilin-1 to the 26S proteasome for proteolytic degradation. Synphilin-1 is an α-synuclein-interacting protein and is a major component of inclusion bodies found in the brains of patients with neurodegenerative α-synucleinopathies, including Parkinson's disease. In our recent studies, we immunostained sections of brains from patients with Parkinson's disease and other α-synucleinopathies and demonstrated that NUB1, as well as synphilin-1, accumulates in the inclusion bodies. To define the role of NUB1 in the formation of these inclusion bodies, we performed a co-transfection assay using cultured HEK293 cells. This assay showed that NUB1 suppresses the formation of synphilin-1-positive inclusions. Further biochemical assays revealed that NUB1 overexpression leads to the proteasomal degradation of synphilin-1. These results and our previous observations suggest that NUB1 indeed targets synphilin-1 to the proteasome for its efficient degradation, which, because of the resultant reduction in synphilin-1, suppresses the formation of synphilin-1-positive inclusions. In addition to these basic science aspects, our findings on NUB1 have two important bearings clinically. First, they suggest that NUB1 could serve as a neuropathological marker in patients with α-synucleinopathies because it is strongly accumulated with synphilin-1 in the inclusions of their brain cells. Second, they suggest that NUB1 could be a potential therapeutic target for α-synucleinopathies.

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Key words: Parkinson's disease; NUB1; NEDD8; ubiquitin; proteasome

Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by the loss of dopaminergic neurons and the presence of ubiquitin-positive cytoplasmic inclusions, called Lewy bodies[1, 2]. Mutations in α-synuclein, parkin, or synphilin-1 have been found in patients with familial or sporadic PD[3-6]. Recently, both α-synuclein and synphilin-1 were shown to be ubiquitinated by E3 ubiquitin ligase activity of wild-type parkin, but not by familial-linked mutant parkin. Moreover, the coexpression of α-synuclein, synphilin-1, and parkin resulted in the formation of Lewy body-like, ubiquitin-positive cytoplasmic inclusions in cultured cells. Importantly, familial-linked mutations in parkin disrupted the formation of the ubiquitin-positive inclusions[7]. These observations indicate that defects in the ubiquitination of Lewy body-associated proteins are involved in the pathogenesis of PD.

During the past 15 years, we have been studying NEDD8[8], which is a ubiquitin-like protein and covalently conjugates to a small number of target proteins to modify their molecular function. We recently identified a novel NEDD8-binding protein, NUB1[9]. Our studies indicated that NUB1 recruits NEDD8 and its conjugates to the S5a subunit of 26S

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proteasome for proteolytic degradation[10]. Thus, NUB1 seems to function as a potent downregulator for NEDD8. Most recently, we performed yeast two-hybrid screening to investigate NUB1-binding proteins. This method isolated synphilin-1 cDNA[11]. Further studies revealed that NUB1 directly interacts with synphilin-1 through its NEDD8-binding site. Furthermore, we found that NUB1 recruits synphilin-1, as well as NEDD8, to the S5a subunit of 26S proteasome for proteolytic degradation[11]. In this review, we described how NUB1 was discovered and then we discussed about recent results to provide a molecular basis for the involvement of NUB1 in the pathogenesis of PD and other α-synucleinopathies. In addition, we discussed about another neurodegenerative disease, Leber congenital amaurosis, which is related to NUB1.

Ubiquitin, NEDD8, and other ubiquitin-like proteins

Ubiquitin is a 76-amino acid protein present in all eukaryotic cells[12]. When this protein covalently conjugates to cellular proteins, it results in an ATP-dependent proteolysis of ubiquitinated proteins by the 26S proteasome in most cases[13] (Fig. 1). Ubiquitin is always synthesized in a precursor form, with one or more amino acids (tail sequence) following a Gly-Gly dipeptide that forms the C-terminus of the mature protein. These tail sequences are cleaved off by ubiquitin C-terminal hydrolase called UCH[14]. After cleavage, the mature form of ubiquitin conjugates to target proteins in the presence of E1 ubiquitin activating enzyme, E2 ubiquitin conjugating enzyme, E3 ubiquitin ligase, and ATP[12]. Several ubiquitin-like proteins have also been reported. These proteins contain one or two ubiquitin-like domains at the N-terminus followed by a cleavable tail sequence at the C-terminus. These ubiquitin-like proteins include ISG15, sumo/sentrin, and NEDD8[8,15-19].

We previously described a human ubiquitin-like protein called sumo-1 (also known as sentrin-1), which can covalently modify a limited number of cellular proteins in a manner analogous to ubiquitin conjugation (ubiquitination)[16,17]. When we searched the database for proteins homologous to sumo-1, we found a sequence for a mouse ubiquitin-like protein, NEDD8, that has a Gly-Gly dipeptide followed by a short-tail sequence at the C-terminus. We provided the first biochemical characterization of the NEDD8 molecule[8]. We showed that the C-terminal tail of NEDD8 is efficiently processed by a C-terminal hydrolase UCH-L3[8,20] and that the mature form of NEDD8 is covalently conjugated to target proteins[8]. Importantly, this NEDD8 conjugation (neddylation) system is found in a broad range of species, including yeast and Arabidopsis[21-23]. So far, NEDD8 has been reported to conjugate a limited number of target proteins, including cullin family members, the von Hippel-Lindau (VHL) tumor suppressor gene product (pVHL), and p53[24-26]. Because neddylation modifies the function of target proteins, the neddylation system seems to regulate many important biological events[25-27].

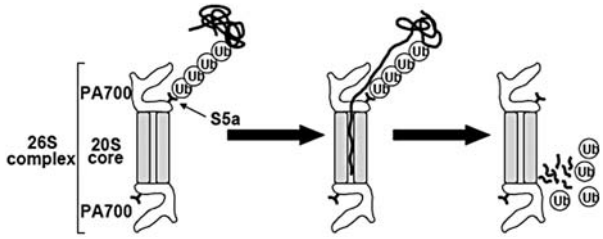

Figure 1 Proteasomal degradation of polyubiquitinated proteins. Polyubiquitinated proteins are captured by S5a subunit of the 26S proteasome, unfoled at PA700, and degraded in the 20S proteasome.

Proteasomal degradation of polyubiquitinated proteins and neddylated proteins

It has been well known that polyubiquitinated proteins are degraded by an ATP-dependent

proteolytic complex called the 26S proteasome[13,28]. It is a large complex (2000 kDa) composed of two 700-kDa subcomplexes, the 20S proteasome and PA700 (19S regulatory complex) (Fig. 1).

The 20S proteasome is composed of a set of small subunits of 21-32 kDa, arranged in a stack of four rings that form a cylinder[13,29] (Fig. 1). This cylindrical structure has a hollow center in which all six catalytic ends are located[30]. The 20S core cylinder is capped at both sides by two large V-shaped terminal modules, named PA700. Thus, the 26S proteasome has a dumbbell-shaped appearance (Fig. 1).

The PA700 complex consists of a set of characteristic heterogeneous subunits of 25-110 kDa. The structure of PA700 can be further subdivided into a base and a lid[29]. The lid is thought to recognize the polyubiquitin chain of ubiquitinated proteins through its subunit, S5a/Rpn10. The ubiquitinated proteins are then unfolded by the base and funneled into the proteolytic 20S core complex for degradation[29] (Fig. 1).

In addition to polyubiquitinated proteins, we recently demonstrated that neddylated proteins are targeted to the 26S proteasome by a NEDD8-interacting protein NUB1 for proteolytic degradation (see below).

NEDD8-interacting protein NUB1

We previously identified a negative regulator of the NEDD8 conjugation system, NUB1, which interacts with NEDD8 and downregulates NEDD8 expression post-translationally[9]. Specifically, we showed that NUB1 possesses a ubiquitin-like domain (UBL) at the N-terminal region and binds to S5a of PA700 of the proteasome[10] (Fig. 2A). Interestingly, a GST pulldown assay revealed that the overexpression of NUB1 leads to a greater precipitation of NEDD8 conjugates with GST-S5a[10], suggesting that NUB1 have an adaptor function between a proteasome subunit S5a and NEDD8 (Fig. 2B). Furthermore, proteasome inhibitors completely block NUB1-mediated down-regulation of NEDD8 expression[10]. These results suggested that NUB1 recruits NEDD8 and its conjugates to the proteasome for degradation, providing a direct functional link between the NEDD8 conjugation system and the proteasomal degradation pathway (Fig. 2B).

NUB1-interacting protein synphilin-1

Synphilin-1, a 919-amino acid protein, is an α-synuclein interacting protein whose function is currently unknown[31]. It is predominantly expressed in neurons, localized in the cytoplasm and presynaptic nerve terminals, and associates with synaptic vesicles. Thus, synphilin-1 is located in the same intracellular compartments as α-synuclein and parkin[31,32]. Pathological studies have shown that synphilin-1 is a component of Lewy bodies in the brains of patients with sporadic PD[33]. Experimental studies have further revealed that the coexpression of synphilin-1 and α-synuclein results in the formation of Lewy body-like cytoplasmic inclusions in cultured cells [31,34]. These observations provide an important basis for the involvement of synphilin-1 in the pathogenesis in PD.

Recently, we found that NUB1 physically interacts with synphilin-1 through its NEDD8-binding site[11], implying that NUB1 also targets synphilin-1 to the proteasome for degradation (Fig. 3). Most recently, we immunostained sections of brains from patients with PD and other α-synucleinopathies and demonstrated that NUB1, as well as synphilin-1, accumulates in the inclusion bodies[11]. To define the role of NUB1 in the formation of these inclusion bodies, we performed a co-transfection assay using cultured HEK293 cells. This assay showed that NUB1 suppresses the formation of synphilin-1-positive inclusions[11]. Further biochemical assays revealed that NUB1 overexpression leads to the proteasomal degradation of synphilin-1[11]. These results and our previous observations suggested

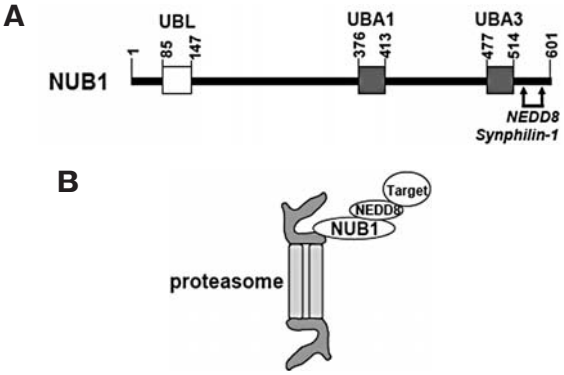

Figure 2 Structure of NUB1. (A) Location of binding site of NEDD8 and synphilin-1. The binding site is indicated by arrows. (B) Relationship between NUB1 and the 26S proteasome.

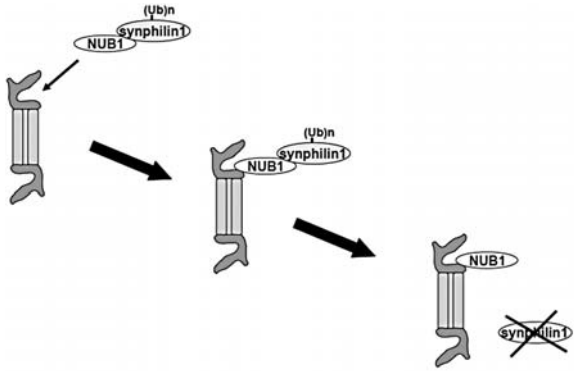

Figure 3 Model for NUB1-mediated targeting of ubiquitinated synphilin-1. In this model, NUB1 efficiently targets synphilin-1 to the 26S proteasome for degradation, resulting in the reduction of synphilin-1-positive inclusions.

that NUB1 indeed targets synphilin-1 to the proteasome for its efficient degradation (Fig. 3), which, because of the resultant reduction in synphilin-1, suppresses the formation of synphilin-1-positive inclusions.

Although we defined that NUB1 promotes the proteasomal degradation of synphilin-1 in cultured cells, the function of NUB1 in human brain has not been elucidated. However, we believe that NUB1 plays the same role in cells of human brain. Because both NUB1 and synphilin-1 are expressed in the normal brain, NUB1 should also promote the proteasomal degradation of synphilin-1 in the cells of normal brain through its interaction with synphilin-1. In the brains of patients with α-synucleinopathies, such as PD, dementia with Lewy bodies (DLB), and multiple system atrophy (MSA), NUB1 should also reduce the expression of synphilin-1 and thereby downregulate the formation of synphilin-1-positive inclusions. Thus, NUB1 seems to play an important role in brain cells under both physiological and pathological conditions.

NUB1 as a pathological marker for α-synucleinopathies

α-synuclein and synphilin-1 are major components of Lewy bodies found in the brains of patients with PD and DLB, as well as of glial cytoplasmic inclusions seen in the brains of patients with MSA[35-37]. Because NUB1 interacts with synphilin-1, we hypothesized that NUB1 is also present in the inclusion bodies in the brains of patients with α-synucleinopathies. To determine this, immunohistochemical investigations were performed on normal brains, brains from patients with α-synucleinopathies (PD, DLB, and MSA), and brains from patients with non-α-synucleinopathies using anti-NUB1 antibody. The anti-NUB1 antibody weakly immunostained the neuronal cytoplasm and processes in the normal brains. Glial cells were not immunolabeled with anti-NUB1 in the normal brains[38]. In PD and DLB, cortical and brainstem-type Lewy bodies were strongly immunolabeled with anti-NUB1[38]. Interestingly, the brainstem-type Lewy bodies showed intense staining in their peripheral portions. In the substantia nigra and locus ceruleus, pale bodies (known as the precursor of Lewy bodies)[39] were also positive for NUB1. Intraneuritic Lewy bodies in the brainstem and peripheral sympathetic ganglia were NUB1-positive. Glial inclusions observed in the midbrain[40] were positive for NUB1. In MSA, in addition to glial cytoplasmic inclusions, anti-NUB1 antibody immunolabeled neuronal

cytoplasmic inclusions, neuronal nuclear inclusions and swollen neurites[38]. Although oligodendroglial nuclear inclusions were occasionally found in sections stained with anti-phosphorylated α-synuclein, no obvious NUB1-positive intranuclear inclusions were noted in the glial cells.

As reported previously, anti-NEDD8 immunolabeled neuronal and glial inclusions in various neurodegenerative diseases[41,42]. In our recent studies[38], however, no NUB1 immunoreactivity was detected in the following structures of non-α-synucleinopathies: neurofibrillary tangles and neuritic plaques in Alzheimer's disease; tau-positive neuronal and glial inclusions in progressive supranuclear palsy, corticobasal degeneration, and Pick's disease; Bunina bodies and skein-like inclusions in amyotrophic lateral sclerosis (ALS); ubiquitinated inclusions in ALS with dementia; and neuronal intranuclear inclusions in Machado-Joseph disease and dentatorubral-pallidoluysian atrophy. Hirano bodies and granulovacuolar degeneration in aged patients were also NUB1 negative[38].

In Fig. 4, we summarized the percent presence of α-synuclein and NUB1 in inclusion bodies of brains[11,38]. In PD, 95% of brainstem-type Lewy bodies were positive for NUB1. In DLB, 82% of cortical Lewy bodies were positive for NUB1. In MSA, 98% of glial cytoplasmic inclusions were positive for NUB1. Thus, the vast majority of inclusions in human α-synucleinopathies contained NUB1. We believe that NUB1 could be detected in all α-synuclein-positive inclusions if a better antibody to NUB1 would be available. In contrast, NUB1 was not detected in pathological inclusions of non-α-synucleinopathies.

Another neurodegenerative disease related to NUB1

Leber congenital amaurosis (LCA) is a genetically heterogeneous, typically autosomal recessive retinal degenerative disease and is responsible for approximately 5% of all inherited retinopathies[43]. LCA is often considered the most severe form of childhood retinopathy, and infants with this disease are usually blind at birth. Previously, Sohocki et al.[44] identified the LCA-associated gene, aryl hydrocarbon receptor-interacting protein-like 1 (AIPL1). Mutations in the AIPL1 gene account for about 7–9% of all LCA cases worldwide and result in autosomal dominant dystrophy of cone–rod photoreceptors[45]. The AIPL1 gene encodes a 384-amino acid protein that contains three tetratricopeptide (TPR) motifs[44,46]. To investigate the function of the AIPL1 protein, we performed yeast two-hybrid cDNA screening of a retinal cDNA library

Neurodegenerative diseases	α-synuclein	NUB1
Parkinson's disease (PD)	100%	95%
Dementia with Lewy bodies (DLB)	100%	82%
Multiple system atrophy (MSA)	100%	98%
Alzheimer's disease (AD)	0%	0%
Pick's disease (PiD)	0%	0%
Progressive supranuclear palsy (PSP)	0%	0%
Corticobasal degeneration (CBD)	0%	0%
Amyotrophic lateral sclerosis (ALS)	0%	0%
Machado-Joseph disease (MJD)	0%	0%
Dentatorubral-pallidoluysian atrophy (DRPLA)	0%	0%

Figure 4 Presence of α-synuclein and NUB1 in inclusion bodies of brains. α-synucleinopathies are shaded, non-α-synucleinopathies are not.

using AIPL1 as bait[47]. This showed that NUB1 strongly interacts with AIPL1. Importantly, previous studies have shown that NUB1 and AIPL1 are expressed within the developing cone and rod photoreceptors and colocalize within the rods of the mature retina[44,47-49]. Recently, we investigated the interaction between NUB1 and AIPL1 mutants associated with LCA and found that this interaction is affected in some AIPL1 mutants (such as W278X, A197P, and C239R), but not in other mutants (such as I206N, G262S, R302L, and P376S)[50]. These results suggested that the abolished interaction between NUB1 and AIPL1 mutants might be involved in the pathogenesis of LCA, but the pathogenesis is not just due to the abolished interaction. Molecules other than NUB1 must also be involved in the pathogenesis.

α-synucleinopathies and LCA as "nubopathies"

So far, no one has reported that LCA is related to α-synucleinopathies. However, because LCA results from degeneration of retinal neurons called photoreceptors, LCA belongs to neurodegenerative diseases, as well as α-synucleinopathies. Interestingly, as described above, LCA and α-synucleinopathies are caused by mutations of NUB1-related proteins AIPL1 and α-synuclein, respectively. Thus, it is possible that NUB1 is involved in the pathogenesis of both neurodegeneration. Therefore, here we propose a new nomenclature "nubopathies" for NUB1-related neurodegenerative diseases such as LCA and α-synucleinopathies. In "nubopathies," we believe that protein complexes containing NUB1 have dysfunction due to a mutation of AIPL1, α-synuclein, or other proteins. To elucidate this, we need to characterize the function of NUB1-containing protein complexes. The outcome of this research will potentially lead to the development of new therapeutic targets for "nubopathies."

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References

1) Lang AE, Lozano AM. Parkinson's disease. First of two parts. N Engl J Med 1998;339:1044-53.

2) Pollanen MS, Dickson DW, Bergeron C. Pathology and biology of the Lewy body. J Neuropathol Exp Neurol 1993;52:183-91.

3) Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 1997;276,2045-7.

4) Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, Przuntek H, Epplen JT, Schols L and Riess O. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. Nat Genet 1998;18:106-8.

5) Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y and Shimizu N. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 1998;392:605-8.

6) Marx FP, Holzmann C, Strauss KM, Li L, Eberhardt O, Gerhardt E, Cookson MR, Hernandez D, Farrer MJ, Kachergus J, Engelender S, Ross CA, Berger K, Schols L, Schulz JB, Riess O and Kruger R. Identification and functional characterization of a novel R621C mutation in the synphilin-1 gene in Parkinson's disease. Hum Mol Genet 2003;12:1223-31.

7) Chung KK, Dawson VL, Dawson TM. The role of the ubiquitin-proteasomal pathway in Parkinson's disease and other neurodegenerative disorders. Trends Neurosci 2001;24:S7-14.

8) Kamitani T, Kito K, Nguyen HP and Yeh ETH. Characterization of NEDD8, a developmentally down-regulated ubiquitin-like molecule. J Biol Chem 1997;272:28557-62.

9) Kito K, Yeh ETH, Kamitani, T. NUB1, a NEDD8-

interacting protein, is induced by interferon and downregulates the NEDD8 expression. J Biol Chem 2001;276:20603-9.

10) Kamitani T, Kito K, Fukuda-Kamitani T, and Yeh ETH. Targeting of NEDD8 and its conjugates for proteasomal degradation by NUB1. J Biol Chem 2001;276:46655-60.

11) Tanji K, Tanaka T, Mori F, Kito K, Takahashi H, Wakabayashi K and Kamitani T. NUB1 suppresses the formation of Lewy body-like inclusions by proteasomal degradation of synphilin-1. Am J Pathol 2006;169:553-65.

12) Hochstrasser M. Ubiquitin-dependent protein degradation. Annu Rev Genet 1996;30:405-39.

13) Coux O, Tanaka K, Goldberg AL. Structure and functions of the 20S and 26S proteasomes. Annu Rev Biochem 1996;65:801-47.

14) Wilkinson KD. Regulation of ubquitin-dependent processes by deubiquitinating enzymes. FASEB J 1997;11:1245-56.

15) Haas AL, Ahrens P, Bright PM, Ankel H. (1987) Interferon induces a 15-kilodalton protein exhibiting marked homology to ubiquitin. J Biol Chem 262:11315-23.

16) Okura T, Gong L, Kamitani T, Wada T, Okura I, Wei C.-F, Chang H.-M, Yeh ETH. Protection against Fas/APO-1- and tumor necrosis factor-mediated cell death by a novel protein, Sentrin. J Immunol 1996;157:4277-81.

17) Kamitani T, Nguyen HP, Yeh ETH. Preferential modification of nuclear proteins by a novel ubiquitin-like molecule. J Biol Chem 1997;272:14001-4.

18) Matunis MJ, Coutavas E, Blobel G. A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. J. Cell Biol 1996;135:1457-70.

19) Mahajan R, Delphin C, Guan T, Gerace L, Melchior F. A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. Cell 1997;88:97-107.

20) Wada H, Kito K, Caskey LS, Yeh ETH, Kamitani T. Cleavage of the C-terminus of NEDD8 by UCH-L3. Biochem Biophys Res Commun 1998;251:688-92.

21) Liakopoulos D, Doenges G, Matuschewski K, Jentsch S. A novel protein modification pathway related to the ubiquitin system. EMBO J 1998; 17:2208-14.

22) Lammer D, Mathias N, Laplaza JM, Jiang W, Liu Y, Callis J, Goebl M, Estelle, M. Modification of yeast Cdc53p by the ubiquitin- related protein Rub 1 p affects function of the SCF^{Cdc4} complex. Genes & Develop 1998;12:914-26.

23) Pozo JC, Timpte C, Tan S, Callis J, Estelle M. The ubiquitin-related protein RUB1 and auxin response in Arabidopsis. Science 1998;280:1760-3.

24) Wada H, Yeh ETH, Kamitani, T. Identification of NEDD8-conjugation site in human cullin-2. Biochem Biophys Res Commun 1999;257:100-5.

25) Stickle NH, Chung J, Klco JM, Hill RP, Kaelin WG Jr, Ohh M. pVHL modification by NEDD8 is required for fibronectin matrix assembly and suppression of tumor development. Mol Cell Biol 2004;24:3251-61.

26) Xirodimas DP, Saville MK, Bourdon JC, Hay RT, Lane DP. Mdm2-mediated NEDD8 conjugation of p53 inhibits its transcriptional activity. Cell 2004; 118:83-97.

27) Yeh ETH, Gong L, Kamitani T. Ubiquitin-like proteins: new wines in new bottles. Gene 2000;248: 1-14.

28) Goldberg AL. The mechanism and functions of ATP-dependent proteases in bacterial and animal cells. Eur J Biochem 1992;203,9-23.

29) Baumeister W, Walz J, Zuhl F, Seemuller E. The proteasome: paradigm of a self-compartmentalizing protease. Cell 1998;92:367-80.

30) Groll M, Ditzel L, Lowe J, Stock D, Bochtler M, Bartunik H, Huber, R. Structure of 20S Proteasome from Yeast at 2.4L Resolution. Nature 1997; 386:463-72.

31) Engelender S, Kaminsky Z, Guo X, Sharp AH, Amaravi RK, Kleiderlein JJ, Margolis RL, Troncoso JC, Lanahan AA, Worley PF, Dawson VL, Dawson TM, Ross CA. Synphilin-1 associates with alpha-synuclein and promotes the formation of cytosolic inclusions. Nat Genet 1999;22:110-4.

32) Ribeiro CS, Carneiro K, Ross CA, Menezes JR,

Engelender S. Synphilin-1 is developmentally localized to synaptic terminals, and its association with synaptic vesicles is modulated by alpha-synuclein. J Biol Chem 2002;277:23927-33.

33) Wakabayashi K, Engelender S, Yoshimoto M, Tsuji S, Ross CA, Takahashi, H. Synphilin-1 is present in Lewy bodies in Parkinson's disease. Ann Neurol 2000;47:521-3.

34) Chung KK, Zhang Y, Lim KL, Tanaka Y, Huang H, Gao J, Ross CA, Dawson VL,Dawson TM. Parkin ubiquitinates the alpha-synuclein-interacting protein, synphilin- 1: implications for Lewy-body formation in Parkinson disease. Nat Med 2001;7:1144-50.

35) Wakabayashi K, Engelender S, Tanaka Y, Yoshimoto M, Mori F, Tsuji S, Ross CA, Takahashi H. Immunocytochemical localization of synphilin-1, an alpha-synuclein- associated protein, in neurodegenerative disorders. Acta Neuropathol (Berl) 2002;103:209-14.

36) Baba M, Nakajo S, Tu PH, Tomita T, Nakaya K, Lee VM, Trojanowski JQ, Iwatsubo T. Aggregation of alpha-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. Am J Pathol 1998;152:879-84.

37) Arima K, Ueda K, Sunohara N, Hirai S, Izumiyama Y, Tonozuka-Uehara H, Kawai, M. Immunoelectron-microscopic demonstration of NACP/alpha-synuclein-epitopes on the filamentous component of Lewy bodies in Parkinson's disease and in dementia with Lewy bodies. Brain Res 1998;808:93-100.

38) Tanji K, Mori F, Kakita A, Zhang H, Kito K, Kamitani T, Takahashi H, Wakabayashi K. Immunohistochemical localization of NUB1, a synphilin-1-binding protein, in neurodegenerative disorders. Acta Neuropathol 2007;114:365-71.

39) Wakabayashi K, Hayashi S, Kakita A, Yamada M, Toyoshima Y, Yoshimoto M, Takahashi H. Accumulation of alpha-synuclein/NACP is a cytopathological feature common to Lewy body disease and multiple system atrophy. Acta Neuropathol (Berl) 1998;96:445-52.

40) Wakabayashi K, Hayashi S, Yoshimoto M, Kudo H, Takahashi H. NACP/alpha- synuclein-positive filamentous inclusions in astrocytes and oligodendrocytes of Parkinson's disease brains. Acta Neuropathol 2000;99:14-20.

41) Kuazi AD, Kito K, Abe Y, Shin R.-W, Kamitani T, Ueda, N. NEDD8 protein is involved in ubiquitinated inclusion bodies. J Pathol 2003;199: 259-66.

42) Mori F, Nishie M, Piao YS, Kito K, Kamitani T, Takahashi H, Wakabayashi K. Accumulation of NEDD8 in neuronal and glial inclusions of neurodegenerative disorders. Neuropathol Appl Neurobiol 2005;31:53-61.

43) Kaplan J, Bonneau D, Frezal J, Munnich A, Dufier JL. Clinical and genetic heterogeneity in retinitis pigmentosa. Hum Genet 1990;85:635-42.

44) Sohocki MM, Bowne SJ, Sullivan LS, Blackshaw S, Cepko CL, Payne AM, Bhattacharya SS, Khaliq S, Qasim Mehdi S, Birch DG, Harrison WR, Elder FF, Heckenlively JR, Daiger SP. Mutations in a new photoreceptor-pineal gene on 17p cause Leber congenital amaurosis. Nat Genet 2000;24:79-83.

45) Sohocki MM, Perrault I, Leroy BP, Payne AM, Dharmaraj S, Bhattacharya SS, Kaplan J, Maumenee IH, Koenekoop R, Meire FM, Birch DG, Heckenlively JR, Daiger SP. Prevalence of AIPL1 mutations in inherited retinal degenerative disease. Mol Genet Metab 2000;70:142-50.

46) Blatch GL, Lassle M. The tetratricopeptide repeat: a structural motif mediating protein-protein interactions. Bioessays 1999;21:932-9.

47) Akey DT, Zhu X, Dyer M, Li A, Sorensen A, Blackshaw S, Fukuda-Kamitani T, Daiger SP, Craft CM, Kamitani T, Sohocki MM. The inherited blindness associated protein AIPL1 interacts with the cell cycle regulator protein NUB1. Hum Mol Genet 2002;11:2723-33.

48) Blackshaw S, Fraioli RE, Furukawa T, Cepko CL. Comprehensive analysis of photoreceptor gene expression and the identification of candidate retinal disease genes. Cell 2001;107:579-89.

49) van der Spuy J, Kim JH, Yu YS, Szel A, Luthert PJ, Clark BJ, Cheetham ME. The expression of the Leber congenital amaurosis protein AIPL1 coincides with rod and cone photoreceptor development. Invest Ophthalmol Vis Sci 2003;44: 5396-403.

50) Kanaya K, Sohocki MM, Kamitani T. Abolished interaction of NUB1 with mutant AIPL1 involved in Leber congenital amaurosis. Biochem Biophys Res Commun 2004;317:768-73.