Ubiquitin ligase Cbl-b and inhibitory Cblin peptides

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ABSTRACT

This review focuses on the Cbl-b muscle atrophy-associated ubiquitin ligase and its inhibitors. Herein, the role of E3 ubiquitin ligase-associated muscle atrophy genes (atrogenes), including MAFbx-1/agrogin-1 and MuRF-1, as well as another ubiquitin ligase, Cbl-b and its inhibitors, is discussed. Cbl-b plays an important role in unloading muscle atrophy caused by spaceflight and in bedridden patients: Cbl-b ubiquitinated and induced the degradation of IRS-1, a key intermediate in the IGF-1 signaling. Furthermore, a pentapetpide (DGpYMP), inhibited Cbl-b-mediated IRS-1 ubiquitination. This peptide-based Cbl-b inhibitor Cblin and its homologous peptides in foods presumably affect muscle atrophy under such conditions.

1. Introduction

Skeletal muscles are the most susceptible organs to the activity of the musculature (mechanical stress) and can vary in volume depending on the degree of physical activity. A marked decrease in muscle mass as a result of unloading, such as during spaceflight or in bedridden individuals, is known as disuse muscle atrophy. We collaborated with Japan Aerospace Exploration Agency (JAXA) to study the changes in atrophied skeletal muscle using the skeletal muscle of rats that had been in space for 2 weeks [1].

The pathways that degrade skeletal muscle constituent proteins include the lysosomal pathway of cathepsins, a calcium-dependent pathway by calpain, and the proteasome pathway, which degrades ubiquitinated proteins. Increased proteasome activity, enhanced ubiquitin expression, and the accumulation of ubiquitinated proteins were observed in skeletal muscles atrophied by spaceflight [1,2], suggesting that the ubiquitin-proteasome protein degradation pathway plays a fundamental role. Briefly, the ubiquitin-proteasome proteolysis pathway consists of the ubiquitination system and 26S proteasome. The ubiquitination system consists of a group of ubiquitin activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3). The 26S proteasome degrades polyubiquitinated substrates in the ubiquitination system (Fig. 1). The expression of ubiquitin ligase, which determines the specificity of the substrate, is the rate-limiting step in this proteolytic pathway. Our comprehensive analysis of ubiquitin ligase expression revealed that Casitas B-lineage lymphoma-b (Cbl-b), muscle RING-finger protein-1 (MuRF-1), Siah-1, and muscle atrophy F-box-1 (MAFbx-1)/atrogin-1 were highly elevated in the atrophied skeletal muscle of the space-flown rats [2]. However, the chip MAFbx-1/atrogin-1 was not used at the time. An analysis of MAFbx-1/atrogin-1 after the publication of the paper showed an approximately 20-fold increase in expression [3,4]. In the first half of this review, muscle atrophy-associated ubiquitin ligases (atrogenes) MAFbx-1/atrogin-1 and MuRF-1 are introduced.

In a previous study, Cbl-b was identified as an upregulated ubiquitin ligase that induces muscle protein degradation [2,5,6]. Cblin, a peptide-based Cbl-b inhibitor, and its N-myristoylated form, suppresses the ubiquitination of insulin receptor substrate-1 (IRS-1), a key adapter protein in insulin-like growth factor-1 (IGF-1) signaling [5,7]. A search for the amino acid sequence of the Cblin peptide in the food protein database resulted in the identification of soy glycinin [8]. In the second half of this review, we describe the effects of the Cblin peptide and Cblin-like peptide in soy glycinin on IRS-1 ubiquitination by Cbl-b.
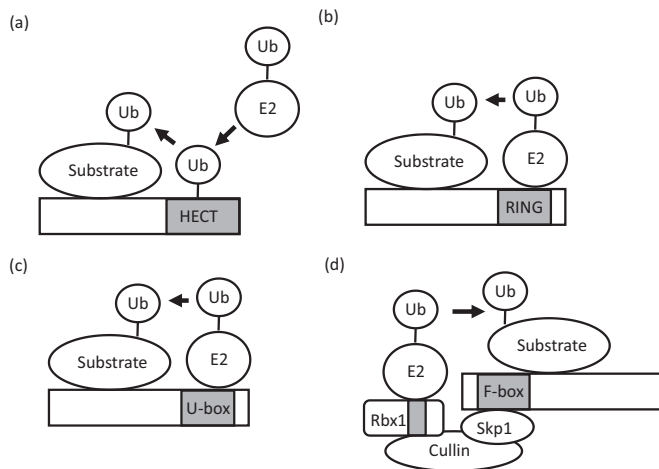
Fig. 1. Types of ubiquitin ligase (E3). (a) HECT-type ubiquitin ligase, (b) RING type ubiquitin ligase, (c) U-box-type ubiquitin ligase, and (d) Cullin-type ubiquitin ligase.

2. Muscle atrophy-associated ubiquitin ligases (atrogenes)

The muscle atrophy genes (atrogenes) MuRF-1 and MAFbx-1/atrogin-1 were identified using the DNA microarray method [3,4]. These genes are part of a group of ubiquitin ligases specific to skeletal muscle and myocardium whose expression increases when the sciatic nerve is resected. Mice with knockouts of these genes are resistant to muscular atrophy due to sciatic nerve resection. The most potent trophic factor for muscle cells is IGF-1. When not present in a muscle atrophy environment, IGF-1 signaling is sufficient to activate muscle protein synthesis and suppress muscle protein degradation [9]. However, under muscle atrophy conditions, IGF-1 signaling is attenuated and Akt-1 activation (phosphorylation) is impaired, resulting in decreased muscle protein synthesis and, conversely, increased muscle protein degradation. Interestingly, the muscle proteolysis and expression of both these atrogenes are regulated by the fork-head box O (FOXO) transcription factor downstream of Akt-1. In other words, when Akt-1 activation decreases, unphosphorylated FOXO transcription factors translocate into the nucleus and increase atrogene transcription.

As shown in Fig. 1, ubiquitin ligase is composed of homologous to the E6-AP c-terminus (HECT), U-box, really interesting new gene (RING), and the Skp1-cullin-F-box (SCF) complex. MAFbx-1/atrogin-1 is a SCF complex-type ubiquitin ligase. SCF complex-type ubiquitin ligases use cullin as a platform protein. The RING domain of a small RING finger protein Rbxl binds to E2, and the F-box protein that binds *via* an adaptor protein Skpl recognizes the substrate protein and ubiquitinates. MAFbx-1/atrogin-1 is a type of F-box protein and has a molecular weight of approximately 40 kDa. Many types of ubiquitin ligases have been reported to bind to other proteins, including neural precursor cell expressed developmentally down-regulated protein 8 (Nedd8)-ubiquitin-like protein and cullin-associated Nedd8-dissociated 1 (CAND1) [10,11].

2.1. MAFbx-1/atrogin-1

MAFbx-1/atrogin-1 is reported to ubiquitinate and degrade myocardial calcineurin A [12]. In fact, the overexpression of MAFbx-1/atrogin-1 inhibits calcineurin-mediated myocardial hypertrophy. However, it remains unclear whether MAFbx-1/atrogin-1 uses calcineurin A as a substrate in skeletal muscle. In skeletal muscle, MyoD, a transcription factor important for muscle cell differentiation and development, interacts with MAFbx-1/atrogin-1 [13]. However, the muscle tissue of MAFbx-1/atrogin-1 deficient mice was found to be normal. The physiological significance of the binding of MAFbx-1/atrogin-1 to MyoD remains unknown. In contrast, it has been reported that the translation system elF3 is a substrate for MAFbx-1/atrogin-1 [14], suggesting it may play an important role in reducing protein synthesis.

2.2. MuRF-1

MuRF-1 is a 40-kDa monomeric RING-type ubiquitin ligase with an E2 binding RING domain at the N-terminus and two coiled-coil domains at the center. Labeit's group identified MuRF-1 as a muscle-specific protein that binds to the giant muscle constituent protein titin (connectin) at the M-band [15,16]. Therefore, MuRF-1 has been suggested to be a ubiquitin ligase for the degradation of skeletal muscle constituent proteins. In fact, MuRF-1 has also been reported to ubiquitinate myosin heavy chains and troponin-1 in skeletal muscle cells and cardiomyocytes [17]. Interestingly, MuRF-1 was also found to be localized in the nucleus and bind to glucocorticoid modulatory element binding protein-1 (GMEB-1), which regulates glucocorticoid-responsive transcription, suggesting that MuRF1 also functions as a transcription factor co-activator (co-repressor) [19].

3. Cbl-b and its inhibitor Cblin

3.1. Cbl-b and unloading muscle atrophy

Like the oncogene c-Cbl, Cbl-b belongs to the Cbl family of adapter proteins, which negatively regulate the action of growth factors and regulate cell differentiation and tissue development. Cbl-b preferentially interacts with the phosphotyrosine of receptors and intracellular signal molecules and promotes their degradation, thereby negatively controlling the action of growth factors. Cbl-b is comprised of a tyrosine kinase binding (TKB) domain at the N-terminal, a RING domain and proline-rich domain at the center, and a leucine zipper at the C-terminal. Proteins that bind to Cbl-b are extremely numerous, although they are not all necessarily ubiquitinated [20].

The ubiquitin ligase Cbl-b plays an important role in skeletal muscle atrophy induced by unloading as a result of bed-rest or microgravity [2,5,6]. The mechanism of Cbl-b-induced muscle atrophy is unique and does not appear to involve the degradation of structural muscle components; rather, it impairs muscular trophic signals in response to unloading conditions. Recent studies investigating the molecular mechanisms of muscle atrophy have found that the role of the IGF-1/phosphoinositide 3-kinase (PI3K)/Akt-1 signaling cascade is a vital pathway in the regulation of the balance between hypertrophy and atrophy [21,22]. These studies suggest that under conditions of muscle wasting, such as disuse, diabetes, and fasting, a decrease in IGF-1/PI3K/Akt-1 signaling enhances the expression of atrogin-1, resulting in muscle atrophy. However, the mechanisms of the unloading-induced impairment in growth factor signaling were still unknown. In a previous study, we reported that under both *in vitro* and *in vivo* experimental conditions, Cbl-b ubiquitinated and induced the specific degradation of IRS-1, a key signaling molecule in skeletal muscle growth, resulting in the down-regulation of the IGF-1 signaling [2,5]. The down-regulation of the IGF-1 signaling stimulated the expression of atrogin-1 through the dephosphorylation (activation) of FOXO3, as well as a reduced mitogen response in the skeletal muscle. Thus, the natural substrate of Cbl-b is IRS-1 in skeletal muscle, and the inactivation of Cbl-b is a beneficial target to prevent muscle atrophy caused by unloading (Fig. 2).

3.2. Identification of Cblin and Cbl-b inhibitory peptides

A pentapeptide (DGpYMP), corresponding to amino acids 606 to 610 in rat IRS-1, inhibited Cbl-b-mediated IRS-1 ubiquitination and strongly decreased the Cbl-b-mediated induction of MAFbx-1/atrogin-1 [2]. This peptide is termed Cblin (abbreviated from Cbl-b inhibitor).
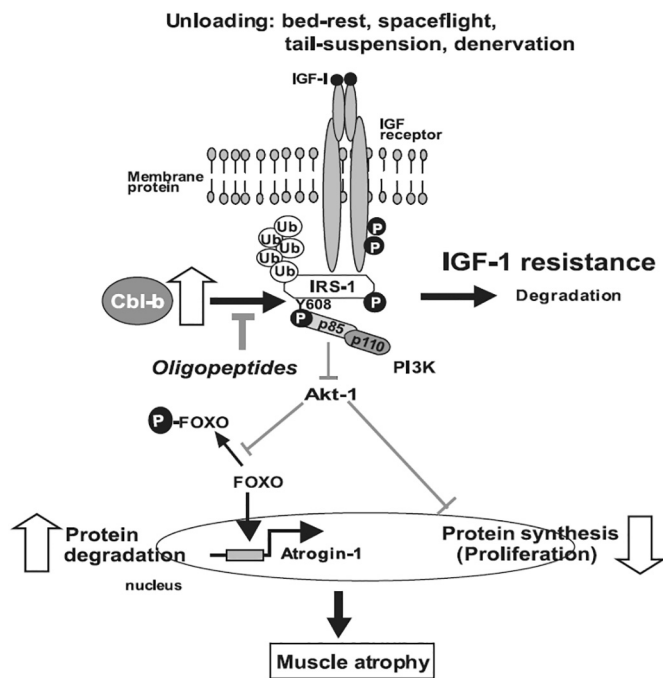
Fig. 2. Mechanistic model of unloading-mediated muscle atrophy. This figure is reproduced from Fig. 7 in Nakao's paper [5] with slight modifications. Unloading induces ubiquitin ligase Cbl-b in myocytes Cbl-b stimulates ubiquitination and the degradation of IRS-1, resulting in IGF-1 resistance in myocytes during unloading. IGF-1 resistance impairs protein synthesis and enhances protein degradation in muscles, resulting in muscle atrophy. Cbl-b and PI3K may competitively interact with IRS-1 at its phosphotyrosine-608. The inhibition of Cbl-b and IRS-1 interaction by Cblin and Cblin-like peptides may restore this impairment of IGF-1 signaling. GSK3, glycogen synthase kinase 3; mTOR, mammalian target of rapamycin; S6K, p70 S6 kinase; Ub, ubiquitin.

Tyrosine-608 of IRS-1 is phosphorylated in response to insulin/IGF-1 [23]. The substitution or dephosphorylation of tyrosine in Cblin failed to prevent Cbl-b-mediated IRS-1 ubiquitination. The replacement of tyrosine-608 with phenylalanine in rat IRS-1 also induced resistance to Cbl-b-mediated ubiquitination. Cblin inhibited the interaction between Cbl-b and IRS-1 through the phosphotyrosine-608 of IRS-1 (Fig. 2). Interestingly, tyrosine-608 is a major docking site for PI3K [23,24]. Thus, Cbl-b may compete with PI3K for binding to IRS-1, and functions as an adapter protein to regulate IGF-1 signaling negatively.

Previous structural and biochemical reports [25,26] indicated that SDGpYTPEPA, a phosphopeptide of ZAP-70, interacted with the TKB domain of c-Cbl, which is highly homologous to Cbl-b. Additionally, the residues that interact with the ZAP-70 peptide [25] are highly

conserved in Cbl ligases. Interestingly, the ZAP-70 peptide very resembled to Cblin. Using the complex structure of the c-Cbl and ZAP-70 peptide as the template, the homology modeling of the Cbl-b TKB domain suggested that the peptide-binding site in the TKB domain was highly conserved in c-Cbl and Cbl-b ubiquitin ligases. The superimposition of the model structure onto the complex structure of c-Cbl and the ZAP-70 peptide also demonstrated a lack of steric hindrance between Cbl-b and the ZAP-70 peptide. On the basis of these observations, it is conceivable that Cbl-b binds to the ZAP-70 peptide and Cblin. It is likely that Cblin acts as a decoy substrate for IRS-1, since Cblin can competitively inhibit the binding of Cbl-b to IRS-1.

3.3. Structure of the TKB domain in Cbl-b complexed with Cblin

Nikawa's group determined the structure of the Cbl-b TKB domain (residues 39–341) co-crystallized with Cblin (refined at 2.5 Å resolution) [27]. The crystallographic asymmetric unit was found to be comprised of three TKB molecules (chains A, B, and C), where only chain B was bound to Cblin. The binding of Cblin to the TKB domain caused a conformational change, resulting in decreased ubiquitin ligase activity. Upon binding to Cblin, the SH2 domain was found to move closer to the 4H domain; this change was previously reported for other substrate-derived phosphopeptide-TKB complexes [27]. The important interaction between Cbl-b and Cblin is the insertion of the phosphorylated tyrosine residue (pY) on Cblin into a positively charged pocket of the Cbl-b TKB domain. This positively charged pocket, which is formed by the residues in the BC loop of the SH2 domain and the AB loop of the 4H domain (Fig. 3), is one of the binding pockets of Cbl-b for substrates with pY. In addition, the Cblin-bound form (chain B) of the Cbl-b TKB domain was similar to the same domain complexed with EGFR- and Zap-70-derived phosphopeptides [28].

The detail interactions (hydrogen bonds and van der Waals interactions) between the Cbl-b TKB domain and Cblin were analyzed using LIGPLOT [29]. The interactions were found to be primarily mediated by 11 hydrogen bonds. The pY of Cblin had six hydrogen-bonds interacting with four residues of the SH2 domain (Arg-286, Ser-288, Cys-289, and Thr-290). Furthermore, the phosphate group of pY formed a water-mediated hydrogen bond with the carbonyl oxygen of Pro-71 on the 4H domain. This water molecule also made hydrogen bonds with the nitrogen backbone of Tyr-266 in the SH2 domain of the Cbl-b TKB domain. Only three additional hydrogen bonds, not involving the pY, were formed between the TKB domain and Cblin residues, suggesting that pY on Cblin is important for its interaction with the Cbl-b TKB domain.

3.4. Modification of the Cblin peptide

Being a pentapeptide, Cblin is easily degraded by aminopeptidase in sera and is difficult to incorporate into the cells. To improve these
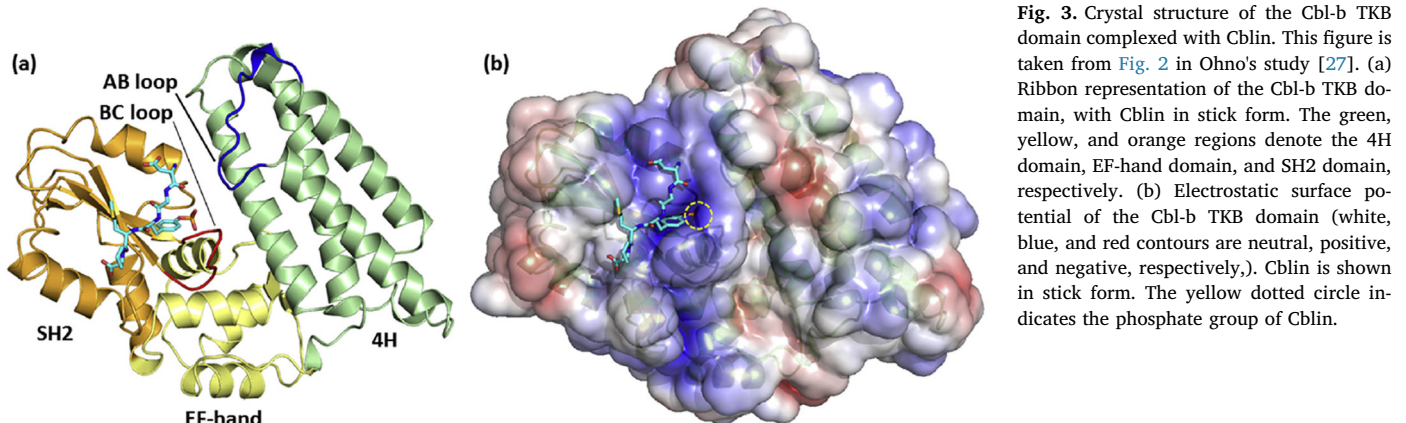

Fig. 3. Crystal structure of the Cbl-b TKB domain complexed with Cblin. This figure is taken from Fig. 2 in Ohno's study [27]. (a) Ribbon representation of the Cbl-b TKB domain, with Cblin in stick form. The green, yellow, and orange regions denote the 4H domain, EF-hand domain, and SH2 domain, respectively. (b) Electrostatic surface potential of the Cbl-b TKB domain (white, blue, and red contours are neutral, positive, and negative, respectively,). Cblin is shown in stick form. The yellow dotted circle indicates the phosphate group of Cblin.

characteristics, we myristoylated the N-termini of the Cblin peptide (C14-Cblin). As expected, N-myristylation increased the permeability of Cblin into the cells about 10-fold, in addition to the resistance against aminopeptidase [7]. When Cblin and C14-Cblin were applied simultaneously to HEK293 cells at the same concentration (50 nmol/dish), the amount of incorporated Cblin and C14-Cblin was 0.30 and 3.6 nmol, respectively. Lastly, the increased permeability of Cblin led to an enhanced inhibition of Cbl-b-mediated IRS-1 ubiquitination [7]. Both Cblin and C14-Cblin similarly and effectively inhibited IRS-1 ubiquitination by Cbl-b in the cell-free ubiquitination assay. However, in another ubiquitination inhibitory assay using Cbl-b- and IRS-1-transfected HEK293 cells, C14-Cblin more effectively inhibited Cbl-b-mediated IRS-1 ubiquitination than Cblin. The concentrations for 50% inhibitory efficacy (IC_{50}) of Cblin and C14-Cblin were 120 and 30 μM, respectively.

3.5. Cblin-like peptide in soy glycinin

As described above, Cblin (DGpYMP) inhibited Cbl-b-mediated ubiquitination and degradation of IRS-1 both in vitro and in vivo [5,7]. We investigated proteins containing a Cblin-like sequence using the Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi/) and discovered that soy glycinin in soy protein contains a sequence similar to that of Cblin peptide, DI/FYNP. Multiple sequence alignments of five soy glycinin homologs, G1, G2, G3, G4, and G, indicated that this sequence, DIYNP, is conserved among these glycinin homologs (Fig. 4) [8]. Since this phosphorylated peptide, DIpYNP, also showed inhibitory activity against Cbl-b-mediated IRS-1 ubiquitination with an IC50 of 350 μM, we called this phosphorylated peptide Cblin-like peptide.

To evaluate the inhibitory effects of dietary soy glycinin on Cbl-b-mediated muscle atrophy in vivo, we administered the four following diets to denervated mice: 20% casein diet (control diet), 20% soy protein isolate (SPI) diet (similar to about 6% glycinin diet), 10% glycinin diet, and 20% glycinin diet [8]. There were no significant differences in the daily intake of food, protein, and energy among the four groups of mice. Denervation for 4 days significantly decreased the cross-sectional area (CSA) of the TA muscle in the control diet group (Fig. 5). Interestingly, the glycinin diet improved this decrease in CSA in proportion to the content of glycinin in the diet. Feeding with 20% soy glycinin diet significantly broadened the CSA distribution compared

to mice on the SPI diet and the 10% soy glycinin +10% casein diet groups. Several studies have found that soy protein is beneficial for preventing muscle damage and muscle protein catabolism after exercise [30,31]. Cblin-like peptides in soy protein may partially contribute to this beneficial effect.

4. Discussion

There is currently no effective drug or food for the prevention of unloading-mediated muscle atrophy. When we first began researching proteases and their inhibitors in Professor Katunuma's lab, he insisted on the importance of developing a useful drug, as well as elucidating the mechanisms of diseases, a philosophy that we have since respected.

Therefore, our aim is to develop Cbl-b inhibitors with small molecular weights (i.e., drugs) against unloading-mediated muscle atrophy. Based on the crystallographic analysis of the complex of Cbl-b TKB domain and Cblin, we are developing chemical Cbl-b inhibitors for use as therapeutic drugs for the treatment and prevention of muscle atrophy.

Because of our expertise in the field of nutrition, we are also interested in functional peptides. Functional peptides are food-derived peptides of nutritional value that exert physiological and hormone-like effects in humans [32]. Functional peptides are inactive within the sequence of their parent protein and can be released by enzymatic hydrolysis during either gastrointestinal digestion or food processing. They are found in milk, egg, meat, and various kinds of fish, as well as in many plants [32]. Since an amino acid sequence similar to Cblin in soy glycinin was found to inhibit the denervation-mediated ubiquitination of IRS-1, this sequence of soy is likely to be a potent functional peptide against muscle atrophy [8]. Unlike various types of medicine, food-derived peptides have few side effects. Based on this property, dietary soy protein is a suitable source of protein for the efficient control of muscle protein catabolism.

However, controversy remains surrounding the detection of food-derived bioactive peptides, unlike other functional substances, such as polyphenols and polyamines. Some peptides found in foods with in vitro biological activity may be further degraded by peptidases during the process of ingestion, digestion, and absorption. In addition, peptides are difficult to absorb in the intestine or other organs. Consequently, these peptides may lose their potential activity when detected based on their
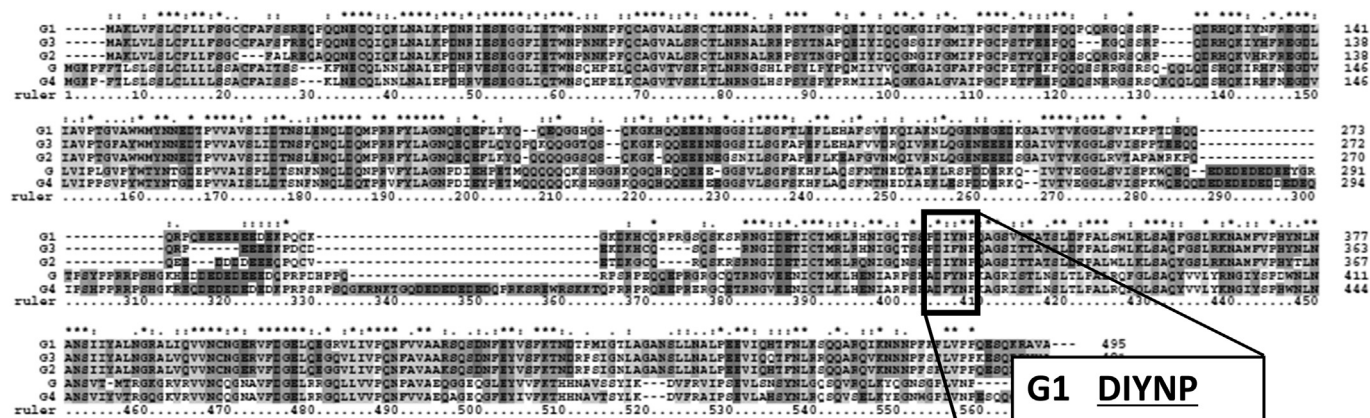

Fig. 4. Amino acid sequence of soy glycinin. This figure is reproduced from Fig. 1 in Abe's paper [8] with modifications. Almost all soy glycinins contain a Cblin-like sequence.
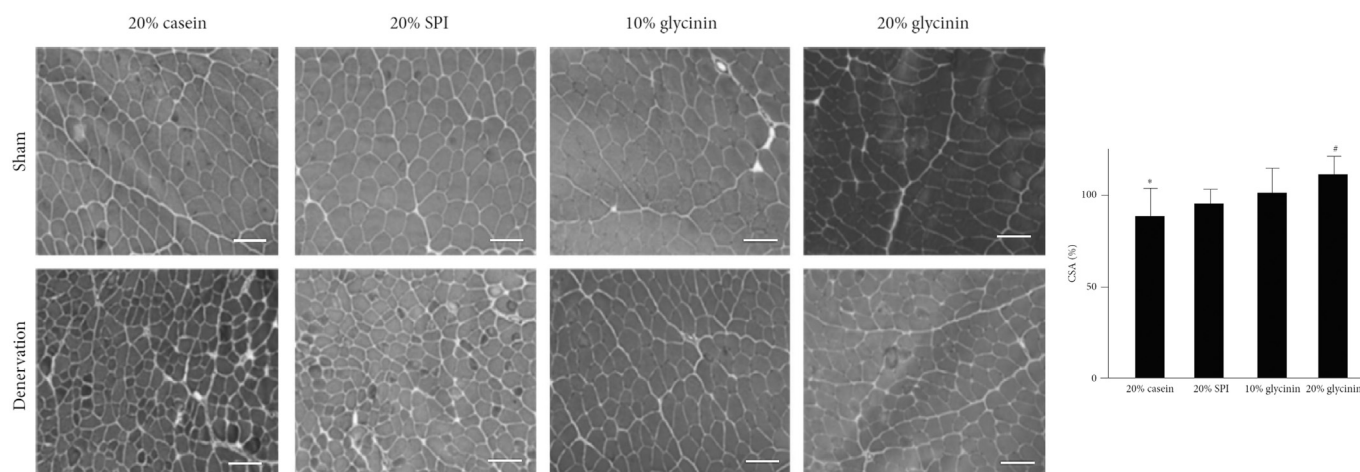
Fig. 5. Effects of dietary soy glycinin protein on CSA in TA of denervated mice. This figure is taken from Fig. 5 in Abe's paper [8]. The right and left legs of C57BL/6 mice were subjected to denervation and sham operation, respectively. Mice were randomly divided into one of the following four diet groups: 20% casein group, 20% soy protein isolate group, 10% soy glycinin + 10% casein group (10% glycinin), and 20% soy glycinin group (20% glycinin). Each diet was started one week before denervation and continued for the duration of the experimental period. Their hind limb muscles were isolated 4 days after denervation. The cross-sectional area (CSA) of myofibers was measured. Scale bar: 100 μm. TA, tibialis anterior muscle.

biological activity. Therefore, the modification of peptides, such as by N-myristylation, may be necessary to fully harness their function. In general, N-myristylation is mediated by fermentation [33]. The fermentation of foods allows for an increase in the permeability and stability of dietary functional peptides.

5. Conclusions

Protease inhibitors are used to treat disused muscle atrophy. Muscle protein degradation increases when muscles are unused under various conditions. In this review, we summarized the structures and functions of ubiquitin ligases associated with muscle atrophy. In particular, the peptide structure-based inhibitors of Cbl-b showed potential as seed compounds for use in medical applications in the future. The Cblin peptide and its derivatives suppress the ubiquitination of IRS-1, a key adapter protein in the IGF-1 signaling pathway, in cell-free ubiquitination assays. The Cblin-like peptide of soy glycinin also suppresses the ubiquitination of IRS-1 in IGF-I-treated HEK293 cells transfected with the expression vectors for Cbl-b, IRS-1, and ubiquitin. Furthermore, soy glycinin-enriched diets affect denervation-induced muscle atrophy. In conclusion, these peptide-based Cbl-b inhibitors represent promising therapeutic strategies for the treatment of muscle atrophy induced under various circumstances, such as in bedridden patients.

Declaration of Competing Interest

The authors declare no conflict of interest.

References

[1] M. Ikemoto, T. Nikawa, S. Takeda, C. Watanabe, T. Kitano, K.M. Baldwin, R. Izumi, I. Nonaka, T. Towatari, S. Teshima, K. Rokutan, K. Kishi, Space shuttle flight (STS-90) enhances degradation of rat myosin heavy chain in association with activation of ubiquitin-proteasome pathway, FASEB J. 15 (2001) 1279–1281.

[2] T. Nikawa, K. Ishidoh, K. Hirasaka, I. Ishihara, M. Ikemoto, M. Kano, E. Kominami, I. Nonaka, T. Ogawa, G.R. Adams, K.M. Baldwin, N. Yasui, K. Kishi, S. Takeda, Skeletal muscle gene expression in space-flown rats, FASEB J. 18 (2004) 522–524.

[3] S.C. Bodine, E. Latres, S. Baumhueter, V.K. Lai, L. Nunez, B.A. Clarke, W.T. Poueymirou, F.J. Panaro, E. Na, K. Dharmarajan, Z.Q. Pan, D.M. Valenzuela, T.M. DeChiara, T.N. Stitt, G.D. Yancopoulos, D.J. Glass, Identification of ubiquitin ligases required for skeletal muscle atrophy, Science 294 (2001) 1704–1708.

[4] M.D. Gomes, S.H. Lecker, R.T. Jagoe, A. Navon, A.L. Goldberg, Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 14440–14445.

[5] R. Nakao, K. Hirasaka, J. Goto, K. Ishidoh, C. Yamada, A. Ohno, Y. Okumura, I. Nonaka, K. Yasutomo, K.M. Baldwin, E. Kominami, A. Higashibata, K. Nagano, K. Tanaka, N. Yasui, E.M. Mills, S. Takeda, T. Ubiquitin ligase Cbl-b is a negative regulator for insulin-like growth factor 1 signaling during muscle atrophy caused by unloading, Mol. Cell. Biol. 29 (2009) 4798–4811.

[6] T. Uchida, Y. Sakashita, K. Kitahata, Y. Yamashita, C. Tomida, Y. Kimori, A. Komatsu, K. Hirasaka, A. Ohno, R. Nakao, A. Higashitani, N. Ishioka, T. Shimazu, T. Kobayashi, Y. Okumura, I. Choi, M. Oarada, E.M. Mills, S. Teshima-Kondo, S. Takeda, E. Tanaka, K. Tanaka, M. Sokabe, T. Nikawa, Reactive oxygen species upregulate expression of muscle atrophy-associated ubiquitin ligase Cbl-b in rat L6 skeletal muscle cells, Am. J. Phys. Cell Phys. 314 (2018) C721–C731.

[7] A. Ochi, T. Abe, R. Nakao, Y. Yamamoto, K. Kitahata, M. Takagi, K. Hirasaka, A. Ohno, S. Teshima-Kondo, G. Taesik, I. Choi, T. Kawamura, H. Nemoto, R. Mukai, J. Terao, T. Nikawa, N-myristoylated ubiquitin ligase Cbl-b inhibitor prevents on glucocorticoid-induced atrophy in mouse skeletal muscle, Arch. Biochem. Biophys. 570 (2015) 23–31.

[8] T. Abe, S. Kohno, T. Yama, A. Ochi, T. Suto, K. Hirasaka, A. Ohno, S. Teshima-Kondo, Y. Okumura, M. Oarada, I. Choi, R. Mukai, J. Terao, T. Nikawa, Soy glycinin contains a functional inhibitory sequence against muscle-atrophy-associated ubiquitin ligase Cbl-b, Int. J. Endocrinol. 2013 (2013) 907565.

[9] M. Sandri, C. Sandri, A. Gilbert, C. Skurk, E. Calabria, A. Picard, K. Walsh, S. Schiaffino, S.H. Lecker, A.L. Goldberg, Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy, Cell 117 (2004) 399–412.

[10] K. Wang, R.J. Deshaies, X. Liu, Assembly and regulation of CRL ubiquitin ligases, Adv. Exp. Med. Biol. 1217 (2020) 33–46.

[11] Y.S. Chua, B.K. Boh, W. Ponyeam, T. Hagen, Regulation of cullin RING E3 ubiquitin ligases by CAND1 in vivo, PLoS One (13) (2011) e16071.

[12] H.H. Li, V. Kedar, C. Zhang, H. McDonough, R. Arya, D.Z. Wang, C. Patterson, Atrogin-1/muscle atrophy F-box inhibits calcineurin-dependent cardiac hypertrophy by participating in an SCF ubiquitin ligase complex, J. Clin. Invest. 114 (2004) 1058–1071.

[13] L.A. Tintignac, J. Lagirand, S. Batonnet, V. Sirri, M.P. Leibovitch, S.A. Leibovitch, Degradation of MyoD mediated by the SCF (MAFbx) ubiquitin ligase, J. Biol. Chem. 280 (2005) 2847–2856.

[14] A. Csibi, M.P. Leibovitch, K. Cornille, L.A. Tintignac, S.A. Leibovitch, MAFbx/Atrogin-1 controls the activity of the initiation factor eIF3-f in skeletal muscle atrophy by targeting multiple C-terminal lysines, J. Biol. Chem. 284 (2009) 4413–4421.

[15] T. Centner, J. Yano, E. Kimura, A.S. McElhinny, K. Pelin, C.C. Witt, M.L. Bang, K. Trombitas, H. Granzier, C.C. Gregorio, H. Sorimachi, S. Labeit, Identification of muscle specific ring finger proteins as potential regulators of the titin kinase

domain, J. Mol. Biol. 306 (2001) 717–726.

[16] B.A. Clarke, D. Drujan, M.S. Willis, L.O. Murphy, R.A. Corpina, E. Burova, S.V. Rakhilin, T.N. Stitt, C. Patterson, E. Latres, D.J. Glass, The E3 ligase MuRF1 degrades myosin heavy chain protein in dexamethasone-treated skeletal muscle, Cell Metab. 6 (2007) 376–385.

[17] V. Kedar, H. McDonough, R. Arya, H.H. Li, H.A. Rockman, C. Patterson, Muscle-specific RING finger 1 is a bona fide ubiquitin ligase that degrades cardiac troponin I, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 18135–18140.

[19] A.S. McElhinny, K. Kakinuma, H. Sorimachi, S. Labeit, C.C. Gregorio, Muscle-specific RING finger-1 interacts with titin to regulate sarcomeric M-line and thick filament structure and may have nuclear functions via its interaction with glucocorticoid modulatory element binding protein-1, J. Cell Biol. 157 (2002) 125–136.

[20] G. Swaminathan, A.Y. Tsygankov, The Cbl family proteins: ring leaders in regulation of cell signaling, J. Cell. Physiol. 209 (2006) 21–43.

[21] D.J. Wells, Improved gene transfer by direct plasmid injection associated with regeneration in mouse skeletal muscle, FEBS Lett. 332 (1993) 179–182.

[22] T.N. Stitt, D. Drujan, B.A. Clarke, F. Panaro, Y. Timofeyva, W.O. Kline, M. Gonzalez, G.D. Yancopoulos, D.J. Glass, The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors, Mol. Cell 14 (2004) 395–403.

[23] S. Boura-Halfon, Y. Zick, Phosphorylation of IRS proteins, insulin action, and insulin resistance, Am. J. Physiol. Endocrinol. Metab. 296 (2009) E581–E591.

[24] D.L. Esposito, Y. Li, A. Cama, M.J. Quon, Tyr (612) and Tyr (632) in human insulin receptor substrate-1 are important for full activation of insulin-stimulated phosphatidylinositol 3-kinase activity and translocation of GLUT4 in adipose cells, Endocrinology 142 (2001) 2833–2840.

[25] W. Meng, S. Sawasdikosol, S.E. Shoelson, M.J. Eck, Structure of the amino-terminal domain of Cbl complexed to its binding site of ZAP-70 kinase, Nature 398 (1999)

84–90.

[26] N. Zheng, P. Wang, P.D. Jeffrey, N.P. Pavletich, Structure of a c-Cbl-UbcH7 complex: RING domain function in ubiquitin-protein ligases, Cell 102 (2000) 533–539.

[27] A. Ohno, A. Ochi, N. Maita, T. Ueji, A. Bando, R. Nakao, K. Hirasaka, T. Abe, S. Teshima-Kondo, H. Nemoto, Y. Okumura, A. Higashibata, S. Yano, H. Tochio, T. Nikawa, Structural analysis of the TKB domain of ubiquitin ligase Cbl-b complexed with its small inhibitory peptide, Cblin, Arch. Biochem. Biophys. 594 (2016) 1–7.

[28] Y. Kobashigawa, A. Tomitaka, H. Kumeta, M. Noda, M. Yamaguchi, F. Inagaki, Autoinhibition and phosphorylation-induced activation mechanisms of human cancer and autoimmune disease-related E3 protein Cbl-b, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 20579–20584.

[29] A.C. Wallace, R.A. Laskowski, J.M. Thornton, LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions, Protein Eng. 8 (1995) 127–134.

[30] K. Masuda, M. Maebuchi, M. Samoto, Y. Ushijima, Y. Uchida, M. Kohno, R. Ito, M. Hirotsuka, Effect of soy-peptide intake on exercise-induced muscle damage, Jpn. J. Clin. Sports Med. 15 (2007) 228–235.

[31] T.G. Anthony, B.J. McDaniel, P. Knoll, P. Bunpo, G.L. Paul, M.A. McNurlan, Feeding meals containing soy or whey protein after exercise stimulates protein synthesis and translation initiation in the skeletal muscle of male rats, J. Nutr. 137 (2007) 357–362.

[32] S. Chakrabarti, S. Guha, K. Majumder, Food-derived bioactive peptides in human health: challenges and opportunities, Nutrients 10 (2018) 1738.

[33] D.R. Johnson, L.J. Knoll, D.E. Levin, J.I. Gordon, Saccharomyces cerevisiae contains four fatty acid activation (FAA) genes: an assessment of their role in regulating protein N-myristoylation and cellular lipid metabolism, J. Cell Biol. 127 (1994) 751–762.