

Review Article

LUBAC: a new player in polyglucosan body disease

Andrew Aboujaoude, Berge Minassian and  Sharmistha Mitra

Division of Neurology, Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX 75390, U.S.A.

Correspondence: Sharmistha Mitra (sharmistha.mitra@utsouthwestern.edu)



Altered protein ubiquitination is associated with the pathobiology of numerous diseases; however, its involvement in glycogen metabolism and associated polyglucosan body (PB) disease has not been investigated in depth. In PB disease, excessively long and less branched glycogen chains (polyglucosan bodies, PBs) are formed, which precipitate in different tissues causing myopathy, cardiomyopathy and/or neurodegeneration. Linear ubiquitin chain assembly complex (LUBAC) is a multi-protein complex composed of two E3 ubiquitin ligases HOIL-1L and HOIP and an adaptor protein SHARPIN. Together they are responsible for M1-linked ubiquitination of substrates primarily related to immune signaling and cell death pathways. Consequently, severe immunodeficiency is a hallmark of many LUBAC deficient patients. Remarkably, all HOIL-1L deficient patients exhibit accumulation of PBs in different organs especially skeletal and cardiac muscle resulting in myopathy and cardiomyopathy with heart failure. This emphasizes LUBAC's important role in glycogen metabolism. To date, neither a glycogen metabolism-related LUBAC substrate nor the molecular mechanism are known. Hence, current reviews on LUBAC's involvement in glycogen metabolism are lacking. Here, we aim to fill this gap by describing LUBAC's involvement in PB disease. We present a comprehensive review of LUBAC structure, its role in M1-linked and other types of atypical ubiquitination, PB pathology in human patients and findings in new mouse models to study the disease. We conclude the review with recent drug developments and near-future gene-based therapeutic approaches to treat LUBAC related PB disease.

Introduction

Protein metabolism involves co-ordinated actions of synthesis and degradation. Ubiquitination is the major mechanism in regulating the degradation. However, it is also a major modifier of protein function regulation in numerous pathways [1,2]. During protein ubiquitination, an 8 kDa protein moiety, ubiquitin is attached to another protein via an enzymatic cascade. First, the enzyme E1 binds to ubiquitin in an ATP dependent manner, which is subsequently transferred to another enzyme E2 and finally, the third enzyme, E3 ubiquitin ligase, attaches the ubiquitin to its substrate [2]. There are three main types of E3 ligases: really interesting new genes (RING) type directly transfers ubiquitin from E2 to substrate; RING between RING (RBR) binds ubiquitin first and then transfers it to the substrate; finally, homologous to E6AP C-terminus (HECT) operates via a mechanism similar to RBR [3] (Figure 1). Irrespective of types, most E3 ligases catalyze the formation of an isopeptide bond between the C-terminus glycine of ubiquitin and a lysine of the substrate and in many cases extends the reaction between ubiquitin molecules to generate polyubiquitin chains [4]. Recently, a protein complex, linear ubiquitin chain assembly complex (LUBAC), consisting of two RBR type of ligases, heme-oxidized IRP2 ubiquitin ligase 1 (HOIL-1L) and HOIL-1L interacting protein (HOIP) and an adapter protein SHANK-associated RH domain interacting protein (SHARPIN) was shown to form an unusual peptide bond between the C-terminal carboxyl group of the donor ubiquitin glycine and the N-terminus amino group of the acceptor ubiquitin methionine, forming a linear polyubiquitin chain [5,6]. The major involvement of such linear ubiquitination has so far been in immune signaling and cell death-related pathways [7,8].

Received: 5 August 2021
Revised: 1 October 2021
Accepted: 4 October 2021

Version of Record published:
28 October 2021

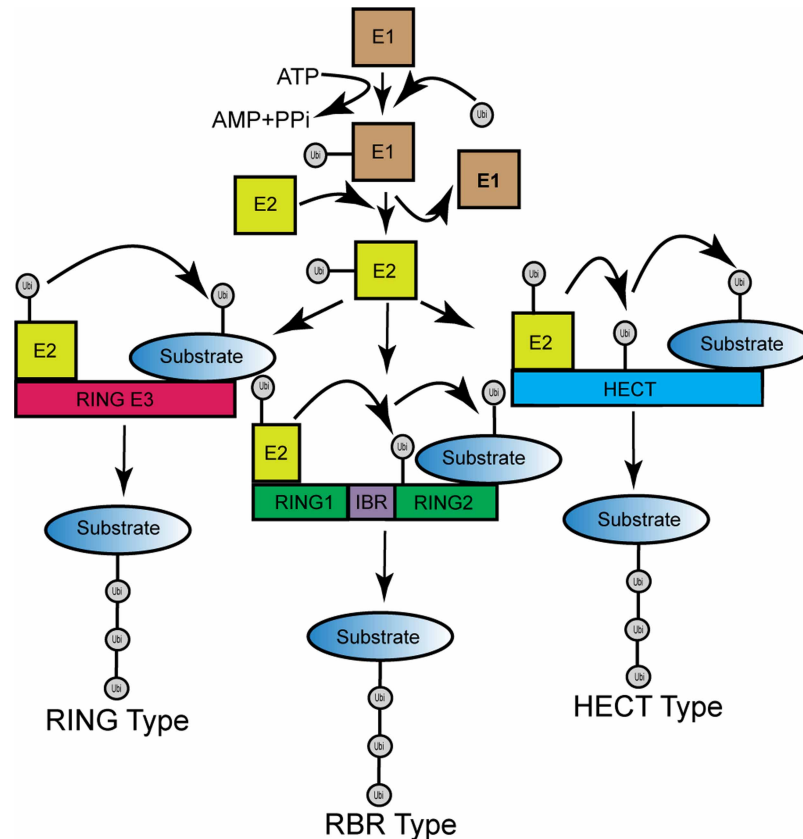


Figure 1. Ubiquitination cascade and types of E3 ubiquitin ligases.

Ubiquitin is transferred from E1 to E2 and finally to one of three types of E3 ubiquitin ligases. The RING-type ligase binds to E2 and directly transfers the ubiquitin from E2 to the substrate. The RBR type has a RING1, RING2 and in-between RING (IBR) domain. The RING1 domain binds to E2-ubiquitin complex and the ubiquitin is transferred to RING2. Finally, the ligase transfers the ubiquitin from RING2 to the substrate. HECT type ligase, similar to RBR, first transfers the ubiquitin from the E2-ubiquitin complex to itself and then to substrate. Ubi, Ubiquitin.

Glycogen, with up to 55 000 glucose units, is the largest molecule in the cytoplasm and serves as a critical energy storage primarily in the liver and muscle but also in the brain, heart and kidney [9,10]. Three Nobel prizes have been awarded in the field of glycogen metabolism and the biology is generally considered well understood. Glycogen synthesis is in particular thought of as settled science resulting from concerted actions between glycogen synthase (GS) which synthesizes glycogen chains and glycogen branching enzyme (GBE). Together, they assure regular branching, and radial, spherical growth of the molecule. Recent discoveries through the genetics of certain rare diseases shed light on additional proteins that operate alongside GS and GBE to ensure correct glycogen architecture [11–15]. In the absence of any of these proteins, some glycogen branches become overlong, driving the affected molecules (now called polyglucosans) to precipitate. Over time, the precipitating molecules accumulate into larger inclusions (PBs) that drive disease in affected organs [16,17]. Intriguingly, all these new proteins that regulate glycogen structure are either E3 ligases or proteins that regulate E3 ligases: (A) Lafora disease (LD) is a fatal progressive myoclonus epilepsy caused by mutations in the *EPM2A* or *EPM2B* genes encoding the proteins laforin and malin, respectively. Malin is a RING-type ligase, which tightly interacts with laforin. Extensive studies with these proteins show that malin's essential function is in glycogen structure regulation, [18,19]. Using *in vitro* experiments, malin was shown to incorporate lysine 48 (K48) or K63-linked ubiquitin chains on substrates leading to their proteasomal or autophagosomal degradation [20]. (B) *HOIL-1L* deficiency results in a disease in which polyglucosans accumulate in skeletal and cardiac muscle, resulting in skeletal and cardiomyopathy and heart failure necessitating cardiac transplantation [21]. While all *HOIL-1L* deficient patients accumulate polyglucosans, some depending on the mutation, also

develop an immunological disorder [22–24]. A glycogen metabolism-related substrate of HOIL-1L is unknown, however, certain proteins are shown to undergo K48-mediated ubiquitination by the ligase followed by proteasomal degradation (reviewed in [25]). Interestingly, a loss of function mutation in HOIP also results in polyglucosans accumulations [26]. (C) Finally, loss of function mutation in *KLHL24* gene leads to polyglucosans accumulations and fatal cardiomyopathy. The *KLHL24* gene encodes KLHL24 which is a constituent of a Cullin-RING E3 ubiquitin ligase complex—CUL3-RBX1-KLHL24 complex, degrades keratin, type I cytoskeletal 14 protein via K48-linked ubiquitination and is involved in fatal epidermolysis bullosa [27,28]. How deficiencies of each of these ubiquitin ligases, and through what substrates and pathways, result in derangement of glycogen structure are not known.

In this review, we focus on LUBAC and its connection to PB disease. First, we describe LUBAC's known structure and function. Next, we detail the polyglucosan pathology of human patients and mouse models. Finally, we review potential therapeutic approaches for affected patients, relying on gene-based information and lessons learned in LD. For reasons mentioned above, this review cannot go far down the molecular pathway from LUBAC deficiency to PB formation, but hopes to serve as a launching point for research in this area.

LUBAC structure and catalysis

Structure of LUBAC

The three components of LUBAC share similarities in domain structures and functions [29,30]. HOIL-1L has an N-terminal LTM followed by UBL domain, an NZF domain and a C-terminal RBR region. The 123 kDa HOIP comprises an N-terminus PUB domain, a classical ZF motif, two NZF domains, two UBA domains (UBA1 and UBA2) and a C-terminal RBR domain followed by a unique region named linear ubiquitin chain determining domain (LDD). SHARPIN is the simplest among the three containing only an N-terminal PH domain followed by LTM, an UBL and NZF domain. The LUBAC complex is formed by the interactions of HOIL-1L and SHARPIN UBLs with UBAs of HOIP [31] as well as by HOIL-1L and SHARPIN's LTMs interactions [32]. A cysteine residue in the RING2 of HOIP together with a C-terminal region makes the LDD and forms the minimal catalytic core for LUBAC [33]. In isolation, this region of HOIP is sufficient forming M1-linked linear ubiquitin chains, although much less efficiently than the RBR-LDD together [34,35].

Role of the two E3 ubiquitin ligases in LUBAC catalysis

HOIP

As stated above, the main catalytic core for M1-linked ubiquitination resides with HOIP's RBR and LDD. A bipartite binding between HOIP-RING1 to E2 and HOIP-IBR to the activated ubiquitin molecule is suitable for transferring the E2-ubiquitin to the RING2 domain [36]. The RING2 domain interacts with the canonical hydrophobic patch and polar arginine residues of the donor ubiquitin. A beta-hairpin located within the LDD further positions the carboxylate group of the C-terminal G76 of the donor ubiquitin towards the catalytic cysteine (C885) of HOIP. The acceptor ubiquitin is locked in place by HOIP's RING2-LDD so that only the N-terminal Met1 and none of the lysines of this ubiquitin molecule are in close proximity to the catalytic cysteine. A conserved histidine residue (H887) of HOIP's RING2-LDD forms a hydrogen bond with M1 of the acceptor ubiquitin and acts as a base to deprotonate and activate the amino group nucleophile of the M1 residue [37].

HOIL-1L

A key question in the LUBAC field is whether there is a catalytic role for HOIL-1L in M1 ubiquitination. While the answer is not completely established, based on recent studies, the following functions of the ligase are starting to emerge:

- (1) *Attaching the first ubiquitin prior to M1-linked chain formation:* In *in vitro* ubiquitination assays, HOIP's RBR-LDD can form free ubiquitin chains. However, only the full-length HOIP/HOIL-1L complex or HOIP-RBR-LDD/HOIL-1 complex is capable of ubiquitinating a physiological protein substrate [34]. Furthermore, full-length HOIL-1L inhibits free ubiquitin chain formation in solution. In fact, mutation in the active site of HOIL-1L (C460A) impairs the ubiquitination of substrate but enhances the formation of free ubiquitin chains by HOIP in solution [34]. These observations suggest that HOIL-1L is critical in targeting the first ubiquitin as well as regulating HOIP's free chain-forming activity at least in *in vitro* setting.

Indeed, in cell cultures, it was shown that the HOIP/HOIL-1L complex has higher affinity for NEMO compared with HOIP alone [38].

- (2) *A potential role in K63/M1 hybrid chain formation?* It was recently reported that most of the M1-linked ubiquitin chains, generated by LUBAC, are hybrids containing both M1 and lysine 63 (K63)-linked polyubiquitin chains [39,40]. Based on these findings, a second mechanism for M1 ubiquitination was proposed, namely that LUBAC substrates are first ubiquitinated by K63-linked small polyubiquitin chains and M1-linked chains are added subsequently. Whether or not HOIL-1L is involved, is not yet known.
- (3) *Unique oxyester linked ubiquitination by HOIL-1L:* The same group as above recently reported that an additional slowly migrating HOIL-1L band on the Western blot is actually a monoubiquitinated species of the protein [41]. It was furthermore shown that this could be hydrolyzed by hydroxylamine, a chemical that selectively cleaves ester bond but not isopeptide (such as K-linked ubiquitination) or peptide (such as M1-linked ubiquitination) bonds. These results suggested that HOIL-1L monoubiquitinates itself through an unusual oxyester linkage (O-linked ubiquitination) [42,43] rather than a canonical lysine-mediated bond. This oxyester-linked monoubiquitinated species of HOIL-1L was enriched within LUBAC. Apart from oxyester-mediated autoubiquitination, it has been suggested that some populations of HOIL-1L substrates also undergo O-linked ubiquitination prior to M1-linked chain attachments [41,44]. An interesting addition came from a recent report, which showed that HOIP produces M1-linked linear ubiquitin chains onto the LUBAC substrate; this is then branched by the HOIL-1L mediated addition of O-linked ubiquitin molecules, suggesting HOIP–HOIL-1L together form a single catalytic center for substrate ubiquitination [45].
- (4) *Monoubiquitination and inhibition of LUBAC by HOIL-1L:* HOIL-1L autoubiquitination was separately reported by a second group [32,46]. Here, the majority of ubiquitinated HOIL-1L species were resistant to hydroxylamine treatment but digested by a deubiquitinase that cleaves K-linked ubiquitin linkages. Their experiments identified ubiquitinated lysine residues of HOIL-1L, HOIP and SHARPIN and a serine residue of HOIP. Strikingly, a mutant HOIL-1L where all the lysine residues were changed to arginine, failed to monoubiquitinate HOIL-1L but increased the activity of LUBAC. Furthermore, HOIL-1L was found to monoubiquitinate HOIP and SHARPIN, priming those HOIP substrates for further linear ubiquitin chain generation. Thus, this HOIL-1L-aided and HOIP-mediated M1-linked ubiquitination of LUBAC components curbs its function.

A multifaceted protein–protein interaction governs LUBAC structure and function

LUBAC is a unique E3 ubiquitin ligase complex, which can determine the type of ubiquitin chain irrespective of which E2 is being used [5]. This is due to a unique interaction between ubiquitin and HOIP's RING2-LDD [33]. Solitarily, HOIP's UBAs inhibits its catalytic activity [5,33,35]. The binding of SHARPIN and HOIL-1L UBLs to HOIP-UBA1 and UBA2, respectively, induces a conformational change within HOIP [31,47]. The RBR and LDD of HOIP are now structurally oriented to facilitate E2 loading and to perform the catalytic reaction. Furthermore, SHARPIN and HOIL-1L interact with each other through LTMs, which folds into a single globular domain upon heterodimerization producing a larger buried surface area than UBA–UBL interactions [32]. This stabilizes the trimeric LUBAC. The UBLs are now secured to HOIP's UBAs, forming a tight complex with the conformation needed for catalysis. Once LUBAC starts chain linkage, these are stabilized by multiple ubiquitin–LUBAC interactions. The purified NZF domain of HOIL-1L binds to M1-linked ubiquitin chains with higher affinity than K-linked chains [48,49]. This might be integral to the retention of LUBAC to the ubiquitinated substrate and the further addition of M1-linked ubiquitins to the substrate. In contrast, HOIP preferentially binds K63-linked ubiquitin oligomers [39]. SHARPIN NZF binds to both K63-linked and M1-linked ubiquitin chains [49,50].

A comprehensive summary on LUBAC catalysis

LUBAC is emerging as an unusual protein complex where two RBR E3 ligases could form multiple unique ubiquitin linkages (O-linked, K-linked and M1-linked) (Figure 2A). Based on the available information, we propose scenarios for LUBAC catalysis (Figure 2B): (i) First, UBLs of SHARPIN and HOIL-1L interact with UBA1 and UBA2 of HOIP, respectively, to remove the auto-inhibitory state of HOIP; (ii) Next, HOIL-1L and SHARPIN interact via the LTMs, forming a stable globular tethering domain that further stabilizes the trimeric LUBAC and orients the complex for E2 recruitment and further catalysis. In this tight complex, a few scenarios

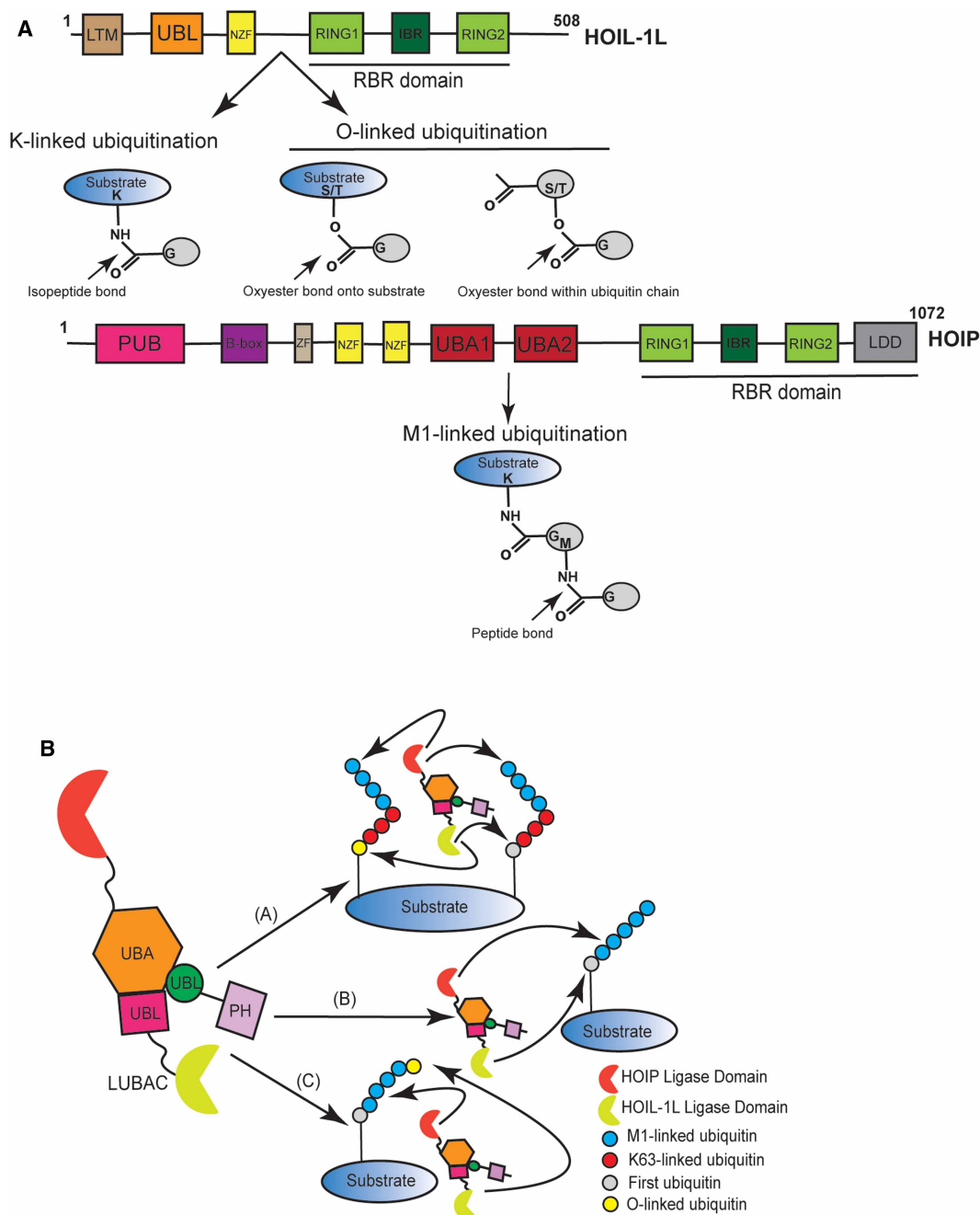


Figure 2. Role of the two E3 ubiquitin ligases in LUBAC catalysis.

Part 1 of 2

(A) Domain architectures of HOIL-1L and HOIP and their ubiquitination reactions. Both HOIL-1L and HOIP have RBR domains. HOIL-1L catalyzes either isopeptide bond formation (with substrate lysine and ubiquitin glycine), producing monoubiquitinated substrates (such as LUBAC components), or an atypical oxyester bond (with substrate/ubiquitin serine/threonine and ubiquitin glycine), generating O-linked substrates or ubiquitin chains. HOIP is strictly engaged in peptide bond formation between methionine and glycine of ubiquitin generating M1-linked polyubiquitin chains. Ubiquitin is represented with gray circles, and residues engaged in bond formation are noted. (B) A comprehensive summary of LUBAC catalysis. After forming the trimeric complex via UBLs–UBA interactions, the ligase domains of HOIP and HOIL-1L can produce heterotypic ubiquitin chains. In scenario A, HOIL-1L can form either an O-linked or a K-linked monoubiquitin bond with the substrate followed by the formation of K63-linked ubiquitin chains by an unknown ligase. HOIP then takes this K63-linked chain as a substrate and attaches M1-linked ubiquitins. In scenario B, HOIL-1L places the first lysine-linked ubiquitin onto the substrate followed by M1-linked chain formation by HOIP. Finally, in scenario C, O-linked ubiquitin chains could be placed by HOIL-1L within M1-linked

Figure 2. Role of the two E3 ubiquitin ligases in LUBAC catalysis.

Part 2 of 2

ubiquitin chain, forming branch points. Future work will establish which of these scenarios is the actual LUBAC mechanism, or whether all three can be utilized depending on substrate.

are possible: (A) an oxyester-linked or lysine-linked ubiquitin bond is formed between the LUBAC substrates and ubiquitin by HOIL-1L, followed by the formation of K63/M1 hybrid chains. *Note:* Which E3 ubiquitin ligase attaches the K-63-linked polyubiquitin chains, is not known; (B) a lysine-linked ubiquitin bond is formed by HOIL-1L, followed by the formation of M1 chains by HOIP; (C) lastly, HOIP directly attaches M1-linked linear ubiquitin chains onto the substrate, which is then branched by HOIL-1L via an oxyester linkage. The tight control of LUBAC's activity comes primarily from two sources: (A) HOIL-1L monoubiquitinates all three components of LUBAC either via the oxyester bond or the lysine-mediated isopeptide bond triggering the M1-linked ubiquitination onto LUBAC subunits and inhibiting its function; (B) LUBAC-specific deubiquitinase OTULIN removes/trims M1 chains on LUBAC subunits, enhancing their function or on certain LUBAC substrates restricting their signaling [51,52]. Similarly, CYLD trims K63 and M1 chains on LUBAC substrates suppressing LUBAC mediated inflammatory pathways [53,54]. Intriguingly, both OTULIN and CYLD bind to the HOIP-PUB domain [55–58]. Therefore, given the complexity, there might be a possibility of their involvement in PB generation as well.

LUBAC and PB disease

Considering the extensive involvement of LUBAC in immune signaling pathways [59], it is not surprising that LUBAC deficient patients and mice show immunodeficiency and autoimmune defects. Intriguingly, mutations in two LUBAC components—*HOIL-1L* and *HOIP* also showed PB accumulation in human patients (described below) or in a mouse model in the case of SHARPIN (our unpublished results).

HOIL-1L deficiency in human

Compared with only two patients with HOIP, multiple patients have been reported with homozygous or compound heterozygous mutations in the *HOIL-1L* gene [21]. OMIM has registered *HOIL-1L* deficiency as PGBM1 WITH OR WITHOUT IMMUNODEFICIENCY (OMIM entry #615895). All patients of PGBM1 exhibit PBs and have skeletal and/or cardiac myopathy, progressive muscle weakness and early disease onset - certain cases even before 1 year of age. So far, one patient has been described with additional mild cognitive impairment and periventricular white matter abnormalities on brain MRI [60]. The histopathological characteristics of PGBM1 differs from PGBM2 (caused by mutation in *GYG1* gene) aiding in differential genetic diagnosis [61]. In contrast with large round, oval-shaped inclusions of PGBM2, PGBM1, shows smaller dotted inclusions within the cytoplasm and depletion of normal glycogen [61]. A recent article potentially explains this observation [62]. The study found that several glycogen metabolism, autophagosome-related and ubiquitin-proteasomal proteins accumulate within the PGBM1 PBs suggesting that sequestration of glycogen metabolism-related enzymes could cause the remarkable decrease in normal glycogen.

A subset of patients suffer immunodeficiency, systemic inflammation of multiple organs and autoinflammation (combined hyper-inflammation and immunodeficiency with early childhood death). It is intriguing that not all patients have the immune pathology. Overall, mutations near the N-terminus lead to the immune dysfunction and PB disease, whereas mutations farther from the N-terminus are associated only with the PB disease [63]. However, there are exceptions, which need to be explained, e.g. the homozygous A18P N-terminus mutation associated with solely PB disease. The explanation can be gleaned by combining the human mutation data with murine results. The first *HOIL-1L* deficient mouse model (*HOIL-1L*^{-/-}) was generated by deletion of exons 7 and 8, which throws the gene's coding sequence out of the frame and generates a premature termination codon. It was anticipated that nonsense-mediated decay (NMD) would completely eliminate the transcript and result in a full knockout. This mouse developed PBs but had limited immunopathology [64]. The second mouse model (*HOIL-1L*^{null/null}) was a true complete knockout, and was embryonic-lethal [32]. The explanation of the difference between the two models proved to be the retention of a smaller transcript sufficient to form LUBAC by LTM mediated interactions [32]. This suggest that LUBAC complex formation, even in small amount, could avert the immune disease, but not the PB disease. Returning to the human situation and the A18P mutation, this mutation sufficiently destabilizes and diminishes LUBAC complexes to induce PB formation, but retains some amount of LUBAC to avoid immune disease.

HOIL-1L deficiency in mice

There are multiple mouse models for HOIL-1L. The first model, as mentioned, had the C-terminal E3 ligase domain of HOIL-1L deleted but expressed a ~30 kDa fragment containing only the N-terminal region [65]. HOIP and SHARPIN protein levels were severely reduced but not completely abolished. Immunologically, these mice were healthy, except for susceptibility to certain bacterial pathogens. Older mice had PB accumulations in multiple organs [64] including the brain (our unpublished results). Complete *HOIL-1L* knockout was generated subsequently [32,66]. This led to embryonic lethality at E10.5 due to endothelial cell death. In a third model, only the first RING1 domain of HOIL-1L was deleted [46]. These mice expressed lower levels of HOIL-1L, but HOIP and SHARPIN were comparable to WT. The LUBAC complex was formed, and M1-linked ubiquitination function was elevated in all tested tissues. Finally, a C458S mutant was generated [41], which inactivates the E3 ligase activity. It will be critical to determine whether these last two mice develop the polyglucosan phenotype.

HOIP deficiency in human

The first patient identified with HOIP deficiency had a homozygous missense mutation in the PUB domain [26]. The affected individual expressed 50% less HOIP mRNA, although no protein was detected by Western blotting. Both HOIL-1L and SHARPIN protein levels were also reduced. LUBAC was not formed as evidenced by co-immunoprecipitation experiments. However, SHARPIN interactions with non-ubiquitinated species of HOIL-1L remained intact. This could be due to the diminished amount of ubiquitinated HOIL-1L in the absence of HOIP, indicating that the amount of HOIP, perhaps influences the ubiquitination states of HOIL-1L as well. The patient accumulated PBs in muscle with lower extremity muscle weakness without any clinical signs of cardiomyopathy. The second patient had two point mutations, which resulted in aberrantly spliced RNA lacking either exon 7 or exon 9 [67]. The peripheral blood mononuclear cells showed reductions in protein amount for full-length HOIP, SHARPIN and HOIL-1L and complete abolition of ubiquitinated HOIL-1L. The interactions between the components of the trimeric LUBAC were also compromised. Muscle biopsy was not performed, and the presence of PBs could, therefore, not be assessed.

HOIP deficiency in other organisms

B cell and T-reg cell-specific deletion of *HOIP* reduced the amounts of HOIL-1L and SHARPIN [68,69]. Similar to *HOIL-1L*, a complete *HOIP* knockout was embryonically lethal between E11.5 and E12.5 due to angiogenic defect [70]. Interestingly, HOIP functions seemed to be important in muscle as indicated by a study with *Drosophila* HOIP ortholog, LUBEL, down-regulation of which caused decreased accumulation of ubiquitinated proteins in ageing muscles [71].

SHARPIN deficiency in mice

Chronic proliferative dermatitis (*cpdm*) was shown to result from a spontaneous mutation in the *Sharpin* gene that deletes a single nucleotide in exon 1 [72]. This results in an early premature stop codon and complete absence of SHARPIN protein. *Sharpin^{cpdm}* pups are viable, however, adults display numerous conditions necessitating euthanasia by 9–12 weeks of age. These include systemic inflammation of multiple organs, severe dermatitis with inflamed and scaly skin, defective lymphoid organ developments in spleen, lymph nodes and absence of Peyer's patches in adult mice [72–74]. In our unpublished work with these mice, we document the presence of PBs in this genotype as well.

A final thought on LUBAC and PB disease

Research in the LUBAC field unveiled its critical role in diverse signaling pathways, especially immune-related ones. However, its function in glycogen metabolism is completely unstudied, though obviously present. One key question is whether HOIL-1L is a main player in this role, which seems to be the case, since all HOIL-1L deficient patients develop PBs. With various possibilities for LUBAC catalysis identified (Figure 2), HOIL-1L's ligase function, previously considered possibly dispensable, is presently emerging front and center in LUBAC function. Hence, identifying new physiological substrates for LUBAC that opens a pathway to glycogen metabolism is now of primary importance. Therefore, the future of LUBAC related PB research should be guided by using existing HOIL-1L mouse models and creating tissue-specific knockout models for HOIP to decipher their involvement in PB pathogenesis.

Therapeutic approaches for LUBAC mediated disorders

In recent years, there has been extensive development of LUBAC inhibitors affecting the linear ubiquitination activity. Despite being effective, they all present pitfalls. BAY 11-7082, a known anti-inflammatory compound inhibits HOIP catalysis possibly by preventing the transfer of ubiquitin from E2 to the catalytic cysteine of the protein, but is non-specific [75]. Gliotoxin, a fungal metabolite, showed great promise; however, had cytotoxic effects [76]. Bendamustine, a drug usually used for leukemia and lymphoma, was effective in suppressing LUBAC activity, but inhibited other E3s as well [77]. Furthermore, these compounds were found to be ineffective in suppressing NF- κ B activation at non-toxic concentrations [78]. The most effective compounds in this regard were HOIPINs, which covalently bind C885 of HOIP and specifically inhibit LUBAC [78,79]. Finally, a series of covalent inhibitors targeting the RBR region of HOIP [80] and peptide inhibitors blocking HOIP/HOIL-1L UBA–UBL interactions showed specificity [81]. Nonetheless, further characterization is warranted. Recently, thiolutin was identified as a potential inhibitor of LUBAC, which inhibited tumor growth in a preclinical mouse model of lymphoma [82].

Future of LUBAC therapeutics: gene therapy

In the absence of any definite treatment and with the advancements of gene therapies for rare diseases in recent years [16,83,84], LUBAC mediated PB disorders could benefit from gene replacement approaches.

Gene replacement therapy

The observation that the polyglucosans phenotype in patients and in mouse models have been primarily associated with mutations in *HOIL-1L* suggests that this gene is an ideal candidate for future targeted gene therapy. The size of the *HOIL-1L* cDNA (2.4 kb) is suitable for packaging in AAV9, presently the flagship vector for gene therapy. Intravenous delivery would be needed to access skeletal muscle and heart. Possibly, even partial expression in cardiac muscle may suffice to rescue a patient's heart, and at least partially improved the skeletal myopathy. AAV9 crosses the blood-brain barrier [85,86] and if there is, any central nervous system disease, so far suggested in one patient, may partially correct the functional deficiency in that organ as well.

Glycogen synthase reduction therapy

Another new therapeutic avenue could be targeting the GS gene for down-regulation. Since polyglucosans, by definition, are glycogen molecules with overlong branches, it was theorized that down-regulating GS activity might correct the problem. This was confirmed in LD mouse models. Down-regulating GS was achieved with (1) crosses between LD models and mice deficient of GS or proteins that activate this enzyme [87–90]; (2) delivering *Gys1* targeting CRISPR/Cas9 to the brain with AAV9 [91] and (3) antisense oligonucleotides (ASO) against GS gene [92]. In all cases, PBs were drastically reduced and consequent neuropathological and behavioral phenotypes improved. Since the basis of this approach is blind to the basic mechanisms of PB formation, the same ASO could be directly repurposed for any PB disease, including *HOIL-1L* deficiency.

Conclusion and future work

Glycogen metabolism in the past, and ubiquitination in the present, are some of the most studied fields of biology. They clearly intersect, including at LUBAC, and when this does not happen, fatal cardio-musculoskeletal and neurological diseases result. Uncovering the ubiquitination pathways and mechanisms in this space will be important for further understanding of the multiple functions of ubiquitination in glycogen metabolism, and their roles in skeletal muscle, heart and brain health. LUBAC is the newest, and possibly most complex, player in ubiquitination. Much about its role in immune pathways has already been uncovered, but multiple other cellular pathways unsurprisingly utilize this intricate machinery. Ongoing deconstruction of the molecular basis of LUBAC, and pathways it regulates, are exciting new frontiers in ubiquitin biology research in health and disease.

Perspective

- The trimeric LUBAC complex is the only E3 ubiquitin ligase complex to employ M1-linked ubiquitin chains onto substrates involved in immune signaling and cell death-related pathways. However, the molecular mechanism that leads to fatal PB pathology in LUBAC deficient patients and mouse models is currently unknown.
- Within the LUBAC complex, HOIP alone is sufficient to form M1-linked ubiquitin chains, but still requires HOIL-1L E3 ligase to place the first O-linked or K-linked ubiquitin onto substrates as well as form O-linked branch points within M1-linked chains. The precise physiological significance of such diverse ubiquitination activity of HOIL-1L is not yet known. However, it is intriguing to observe that HOIL-1L deficiency in human and mouse models, in all cases, causes the PB phenotype suggesting the importance of this ligase in glycogen metabolism.
- To understand the role of LUBAC in glycogen metabolism, conditional knockout mouse models for HOIL-1L and HOIP should be used to identify glycogen metabolism-related physiological substrates of this complex. Until better understanding is acquired, gene-based therapies could be employed to help patients with disease resulting from LUBAC deficiency.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

This work was funded by the National Institutes of Health under award P01NS097197 to B.M. and Dr. Roy Elterman Pediatric Neurology Research Fund awarded to S.M.

Open Access

Open access for this article was enabled by the participation of University of Texas Southwestern Medical Center in an all-inclusive *Read & Publish* pilot with Portland Press and the Biochemical Society under a transformative agreement with EBSCO.

Authors Contribution

A.A., B.M. and S.M. wrote and edited the manuscript.

Abbreviations

CUL3, Cullin 3; HOIPINs, HOIP inhibitors; KLHL24, Kelch like protein 24; LTM, LUBAC tethering motif; LUBEL, linear ubiquitin E3 ligase; NEMO, NF- κ B essential modulator; NF- κ B, nuclear factor Kappa B; NZF, Npl4-type zinc finger; OTULIN, OTU deubiquitinase with linear linkage specificity; PH, pleckstrin homology; PUB, PNGase and UBA or UBX containing protein; UBA, ubiquitin associated; UBL, ubiquitin like; ZF, zinc finger.

References

- 1 Oh, E., Akopian, D. and Rape, M. (2018) Principles of ubiquitin-dependent signaling. *Annu. Rev. Cell Dev. Biol.* **34**, 137–162 <https://doi.org/10.1146/annurev-cellbio-100617-062802>
- 2 Swatek, K.N. and Komander, D. (2016) Ubiquitin modifications. *Cell Res.* **26**, 399–422 <https://doi.org/10.1038/cr.2016.39>
- 3 Cotton, T.R. and Lechtenberg, B.C. (2020) Chain reactions: molecular mechanisms of RBR ubiquitin ligases. *Biochem. Soc. Trans.* **48**, 1737–1750 <https://doi.org/10.1042/BST20200237>
- 4 Deol, K.K., Lorenz, S. and Strieter, E.R. (2019) Enzymatic logic of ubiquitin chain assembly. *Front. Physiol.* **10**, 835 <https://doi.org/10.3389/fphys.2019.00835>
- 5 Kirisako, T., Kamei, K., Murata, S., Kato, M., Fukumoto, H., Kanie, M. et al. (2006) A ubiquitin ligase complex assembles linear polyubiquitin chains. *EMBO J.* **25**, 4877–4887 <https://doi.org/10.1038/sj.emboj.7601360>
- 6 Rittinger, K. and Ikeda, F. (2017) Linear ubiquitin chains: enzymes, mechanisms and biology. *Open Biol.* **7**, 170026 <https://doi.org/10.1098/rsob.170026>
- 7 Hrdinka, M. and Gyrd-Hansen, M. (2017) The Met1-linked ubiquitin machinery: emerging themes of (de)regulation. *Mol. Cell* **68**, 265–280 <https://doi.org/10.1016/j.molcel.2017.09.001>

- 8 Dittmar, G. and Winklhofer, K.F. (2019) Linear ubiquitin chains: cellular functions and strategies for detection and quantification. *Front. Chem.* **7**, 915 <https://doi.org/10.3389/fchem.2019.00915>
- 9 Calder, P.C. (1991) Glycogen structure and biogenesis. *Int. J. Biochem.* **23**, 1335–1352 [https://doi.org/10.1016/0020-711X\(91\)90274-Q](https://doi.org/10.1016/0020-711X(91)90274-Q)
- 10 Adeva-Andary, M.M., Gonzalez-Lucan, M., Donapetry-Garcia, C., Fernandez-Fernandez, C. and Ameneiros-Rodriguez, E. (2016) Glycogen metabolism in humans. *BBA Clin.* **5**, 85–100 <https://doi.org/10.1016/j.bbacli.2016.02.001>
- 11 Minassian, B.A., Lee, J.R., Herbrick, J.A., Huizenga, J., Soder, S., Mungall, A.J. et al. (1998) Mutations in a gene encoding a novel protein tyrosine phosphatase cause progressive myoclonus epilepsy. *Nat. Genet.* **20**, 171–174 <https://doi.org/10.1038/2470>
- 12 Tagliabracchi, V.S., Turnbull, J., Wang, W., Girard, J.M., Zhao, X., Skurat, A.V. et al. (2007) Laforin is a glycogen phosphatase, deficiency of which leads to elevated phosphorylation of glycogen in vivo. *Proc. Natl Acad. Sci. U.S.A.* **104**, 19262–19266 <https://doi.org/10.1073/pnas.0707952104>
- 13 Gentry, M.S., Downen, III, R.H., Worby, C.A., Mattoo, S., Ecker, J.R. and Dixon, J.E. (2007) The phosphatase laforin crosses evolutionary boundaries and links carbohydrate metabolism to neuronal disease. *J. Cell Biol.* **178**, 477–488 <https://doi.org/10.1083/jcb.200704094>
- 14 Chan, E.M., Young, E.J., Lanzano, L., Munteanu, I., Zhao, X., Christopoulos, C.C. et al. (2003) Mutations in NHLRC1 cause progressive myoclonus epilepsy. *Nat. Genet.* **35**, 125–127 <https://doi.org/10.1038/ng1238>
- 15 Roach, P.J., Depaoli-Roach, A.A., Hurley, T.D. and Tagliabracchi, V.S. (2012) Glycogen and its metabolism: some new developments and old themes. *Biochem. J.* **441**, 763–787 <https://doi.org/10.1042/BJ20111416>
- 16 Mitra, S., Gumusgoz, E. and Minassian, B.A. (2021) Lafora disease: current biology and therapeutic approaches. *Rev. Neurol. (Paris)* <https://doi.org/10.1016/j.neuro.2021.06.006>
- 17 Nitschke, F., Ahonen, S.J., Nitschke, S., Mitra, S. and Minassian, B.A. (2018) Lafora disease - from pathogenesis to treatment strategies. *Nat. Rev. Neurol.* **14**, 606–617 <https://doi.org/10.1038/s41582-018-0057-0>
- 18 Nitschke, F., Sullivan, M.A., Wang, P., Zhao, X., Chown, E.E., Perri, A.M. et al. (2017) Abnormal glycogen chain length pattern, not hyperphosphorylation, is critical in Lafora disease. *EMBO Mol. Med.* **9**, 906–917 <https://doi.org/10.15252/emmm.201707608>
- 19 Sullivan, M.A., Nitschke, S., Steup, M., Minassian, B.A. and Nitschke, F. (2017) Pathogenesis of Lafora disease: transition of soluble glycogen to insoluble polyglucosan. *Int. J. Mol. Sci.* **18**, 1743 <https://doi.org/10.3390/ijms18081743>
- 20 Garcia-Gimeno, M.A., Knecht, E. and Sanz, P. (2018) Lafora disease: a ubiquitination-related pathology. *Cells* **7**, 87 <https://doi.org/10.3390/cells7080087>
- 21 Phadke, R., Hedberg-Oldfors, C., Scalco, R.S., Lowe, D.M., Ashworth, M., Novelli, M. et al. (2020) RBCK1-related disease: a rare multisystem disorder with polyglucosan storage, auto-inflammation, recurrent infections, skeletal, and cardiac myopathy-Four additional patients and a review of the current literature. *J. Inher. Metab. Dis.* **43**, 1002–1013 <https://doi.org/10.1002/jimd.12234>
- 22 Boisson, B., Laplantine, E., Prando, C., Giliani, S., Israelsson, E., Xu, Z. et al. (2012) Immunodeficiency, autoinflammation and amylopectinosis in humans with inherited HOIL-1 and LUBAC deficiency. *Nat. Immunol.* **13**, 1178–1186 <https://doi.org/10.1038/ni.2457>
- 23 Nilsson, J., Schoser, B., Laforet, P., Kalev, O., Lindberg, C., Romero, N.B. et al. (2013) Polyglucosan body myopathy caused by defective ubiquitin ligase RBCK1. *Ann. Neurol.* **74**, 914–919 <https://doi.org/10.1002/ana.23963>
- 24 Wang, K., Kim, C., Bradfield, J., Guo, Y., Toskala, E., Otieno, F.G. et al. (2013) Whole-genome DNA/RNA sequencing identifies truncating mutations in RBCK1 in a novel Mendelian disease with neuromuscular and cardiac involvement. *Genome Med.* **5**, 67 <https://doi.org/10.1186/gm471>
- 25 Elton, L., Carpentier, I., Verhelst, K., Staal, J. and Beyaert, R. (2015) The multifaceted role of the E3 ubiquitin ligase HOIL-1: beyond linear ubiquitination. *Immunol. Rev.* **266**, 208–221 <https://doi.org/10.1111/immr.12307>
- 26 Boisson, B., Laplantine, E., Dobbs, K., Cobat, A., Tarantino, N., Hazen, M. et al. (2015) Human HOIP and LUBAC deficiency underlies autoinflammation, immunodeficiency, amylopectinosis, and lymphangiectasia. *J. Exp. Med.* **212**, 939–951 <https://doi.org/10.1084/jem.20141130>
- 27 Hedberg-Oldfors, C., Abramsson, A., Osborn, D.P.S., Danielsson, O., Fazlinezhad, A., Nilipour, Y. et al. (2019) Cardiomyopathy with lethal arrhythmias associated with inactivation of KLHL24. *Hum. Mol. Genet.* **28**, 1919–1929 <https://doi.org/10.1093/hmg/ddz032>
- 28 Lin, Z., Li, S., Feng, C., Yang, S., Wang, H., Ma, D. et al. (2016) Stabilizing mutations of KLHL24 ubiquitin ligase cause loss of keratin 14 and human skin fragility. *Nat. Genet.* **48**, 1508–1516 <https://doi.org/10.1038/ng.3701>
- 29 Spit, M., Rieser, E. and Walczak, H. (2019) Linear ubiquitination at a glance. *J. Cell Sci.* **132**, jcs208512 <https://doi.org/10.1242/jcs.208512>
- 30 Sasaki, K. and Iwai, K. (2015) Roles of linear ubiquitynylation, a crucial regulator of NF-kappaB and cell death, in the immune system. *Immunol. Rev.* **266**, 175–189 <https://doi.org/10.1111/immr.12308>
- 31 Yagi, H., Ishimoto, K., Hiromoto, T., Fujita, H., Mizushima, T., Uekusa, Y. et al. (2012) A non-canonical UBA-UBL interaction forms the linear-ubiquitin-chain assembly complex. *EMBO Rep.* **13**, 462–468 <https://doi.org/10.1038/embor.2012.24>
- 32 Fujita, H., Tokunaga, A., Shimizu, S., Whiting, A.L., Aguilar-Alonso, F., Takagi, K. et al. (2018) Cooperative domain formation by homologous motifs in HOIL-1L and SHARPIN plays a crucial role in LUBAC stabilization. *Cell Rep.* **23**, 1192–1204 <https://doi.org/10.1016/j.celrep.2018.03.112>
- 33 Smit, J.J., Monteferrario, D., Noordermeer, S.M., van Dijk, W.J., van der Reijden, B.A. and Sixma, T.K. (2012) The E3 ligase HOIP specifies linear ubiquitin chain assembly through its RING-IBR-RING domain and the unique LDD extension. *EMBO J.* **31**, 3833–3844 <https://doi.org/10.1038/emboj.2012.217>
- 34 Smit, J.J., van Dijk, W.J., El Atmioui, D., Merckx, R., Ovaas, H. and Sixma, T.K. (2013) Target specificity of the E3 ligase LUBAC for ubiquitin and NEMO relies on different minimal requirements. *J. Biol. Chem.* **288**, 31728–31737 <https://doi.org/10.1074/jbc.M113.495846>
- 35 Stieglitz, B., Morris-Davies, A.C., Koliopoulos, M.G., Christodoulou, E. and Rittinger, K. (2012) LUBAC synthesizes linear ubiquitin chains via a thioester intermediate. *EMBO Rep.* **13**, 840–846 <https://doi.org/10.1038/embor.2012.105>
- 36 Lechtenberg, B.C., Rajput, A., Sanishvili, R., Dobaczewska, M.K., Ware, C.F., Mace, P.D. et al. (2016) Structure of a HOIP/E2~ubiquitin complex reveals RBR E3 ligase mechanism and regulation. *Nature* **529**, 546–550 <https://doi.org/10.1038/nature16511>
- 37 Stieglitz, B., Rana, R.R., Koliopoulos, M.G., Morris-Davies, A.C., Schaeffer, V., Christodoulou, E. et al. (2013) Structural basis for ligase-specific conjugation of linear ubiquitin chains by HOIP. *Nature* **503**, 422–426 <https://doi.org/10.1038/nature12638>
- 38 Tokunaga, F. and Iwai, K. (2012) Linear ubiquitination: a novel NF-kappaB regulatory mechanism for inflammatory and immune responses by the LUBAC ubiquitin ligase complex. *Endocr. J.* **59**, 641–652 <https://doi.org/10.1507/endocrj.EJ12-0148>
- 39 Emmerich, C.H., Ordureau, A., Strickson, S., Arthur, J.S., Pedrioli, P.G., Komander, D. et al. (2013) Activation of the canonical IKK complex by K63/M1-linked hybrid ubiquitin chains. *Proc. Natl Acad. Sci. U.S.A.* **110**, 15247–15252 <https://doi.org/10.1073/pnas.1314715110>

- 40 Emmerich, C.H., Bakshi, S., Kelsall, I.R., Ortiz-Guerrero, J., Shpiro, N. and Cohen, P. (2016) Lys63/Met1-hybrid ubiquitin chains are commonly formed during the activation of innate immune signalling. *Biochem. Biophys. Res. Commun.* **474**, 452–461 <https://doi.org/10.1016/j.bbrc.2016.04.141>
- 41 Kelsall, I.R., Zhang, J., Knebel, A., Arthur, J.S.C. and Cohen, P. (2019) The E3 ligase HOIL-1 catalyses ester bond formation between ubiquitin and components of the Myddosome in mammalian cells. *Proc. Natl Acad. Sci. U.S.A.* **116**, 13293–13298 <https://doi.org/10.1073/pnas.1905873116>
- 42 Wang, X., Herr, R.A. and Hansen, T.H. (2012) Ubiquitination of substrates by esterification. *Traffic* **13**, 19–24 <https://doi.org/10.1111/j.1600-0854.2011.01269.x>
- 43 Pao, K.C., Wood, N.T., Knebel, A., Rafie, K., Stanley, M., Mabbitt, P.D. et al. (2018) Activity-based E3 ligase profiling uncovers an E3 ligase with esterification activity. *Nature* **556**, 381–385 <https://doi.org/10.1038/s41586-018-0026-1>
- 44 Cohen, P., Kelsall, I.R., Nanda, S.K. and Zhang, J. (2020) HOIL-1, an atypical E3 ligase that controls MyD88 signalling by forming ester bonds between ubiquitin and components of the Myddosome. *Adv. Biol. Regul.* **75**, 100666 <https://doi.org/10.1016/j.jbior.2019.100666>
- 45 Rodriguez Carvajal, A., Grishkovskaya, I., Gomez Diaz, C., Vogel, A., Sonn-Segev, A., Kushwah, M.S. et al. (2021) The linear ubiquitin chain assembly complex (LUBAC) generates heterotypic ubiquitin chains. *eLife* **10**, e60660 <https://doi.org/10.7554/eLife.60660>
- 46 Fuseya, Y., Fujita, H., Kim, M., Ohtake, F., Nishide, A., Sasaki, K. et al. (2020) The HOIL-1L ligase modulates immune signalling and cell death via monoubiquitination of LUBAC. *Nat. Cell Biol.* **22**, 663–673 <https://doi.org/10.1038/s41556-020-0517-9>
- 47 Liu, J., Wang, Y., Gong, Y., Fu, T., Hu, S., Zhou, Z. et al. (2017) Structural insights into SHARPIN-mediated activation of HOIP for the linear ubiquitin chain assembly. *Cell Rep.* **21**, 27–36 <https://doi.org/10.1016/j.celrep.2017.09.031>
- 48 Sato, Y., Fujita, H., Yoshikawa, A., Yamashita, M., Yamagata, A., Kaiser, S.E. et al. (2011) Specific recognition of linear ubiquitin chains by the Npl4 zinc finger (NZF) domain of the HOIL-1L subunit of the linear ubiquitin chain assembly complex. *Proc. Natl Acad. Sci. U.S.A.* **108**, 20520–20525 <https://doi.org/10.1073/pnas.1109088108>
- 49 Shimizu, S., Fujita, H., Sasaki, Y., Tsuruyama, T., Fukuda, K. and Iwai, K. (2016) Differential involvement of the Npl4 zinc finger domains of SHARPIN and HOIL-1L in linear ubiquitin chain assembly complex-mediated cell death protection. *Mol. Cell Biol.* **36**, 1569–1583 <https://doi.org/10.1128/MCB.01049-15>
- 50 Ikeda, F., Deribe, Y.L., Skanland, S.S., Stieglitz, B., Grabbe, C., Franz-Wachtel, M. et al. (2011) SHARPIN forms a linear ubiquitin ligase complex regulating NF-kappaB activity and apoptosis. *Nature* **471**, 637–641 <https://doi.org/10.1038/nature09814>
- 51 Heger, K., Wickliffe, K.E., Ndoja, A., Zhang, J., Murthy, A., Dugger, D.L. et al. (2018) OTULIN limits cell death and inflammation by deubiquitinating LUBAC. *Nature* **559**, 120–124 <https://doi.org/10.1038/s41586-018-0256-2>
- 52 Weinelt, N. and van Wijk, S.J.L. (2021) Ubiquitin-dependent and -independent functions of OTULIN in cell fate control and beyond. *Cell Death Differ.* **28**, 493–504 <https://doi.org/10.1038/s41418-020-00675-x>
- 53 Hrdinka, M., Füll, B.K., Zucca, M., Leske, D., Bagola, K., Yabal, M. et al. (2016) CYLD limits Lys63- and Met1-linked ubiquitin at receptor complexes to regulate innate immune signaling. *Cell Rep.* **14**, 2846–2858 <https://doi.org/10.1016/j.celrep.2016.02.062>
- 54 Takiuchi, T., Nakagawa, T., Tamiya, H., Fujita, H., Sasaki, Y., Saeki, Y. et al. (2014) Suppression of LUBAC-mediated linear ubiquitination by a specific interaction between LUBAC and the deubiquitinases CYLD and OTULIN. *Genes Cells* **19**, 254–272 <https://doi.org/10.1111/gtc.12128>
- 55 Schaeffer, V., Akutsu, M., Olma, M.H., Gomes, L.C., Kawasaki, M. and Dikic, I. (2014) Binding of OTULIN to the PUB domain of HOIP controls NF-kappaB signaling. *Mol. Cell* **54**, 349–361 <https://doi.org/10.1016/j.molcel.2014.03.016>
- 56 Elliott, P.R., Nielsen, S.V., Marco-Casanova, P., Füll, B.K., Keusekotten, K., Mailand, N. et al. (2014) Molecular basis and regulation of OTULIN-LUBAC interaction. *Mol. Cell* **54**, 335–348 <https://doi.org/10.1016/j.molcel.2014.03.018>
- 57 Kupka, S., De Miguel, D., Draber, P., Martino, L., Surinova, S., Rittinger, K. et al. (2016) SPATA2-mediated binding of CYLD to HOIP enables CYLD recruitment to signaling complexes. *Cell Rep.* **16**, 2271–2280 <https://doi.org/10.1016/j.celrep.2016.07.086>
- 58 Elliott, P.R., Leske, D., Hrdinka, M., Bagola, K., Füll, B.K., McLaughlin, S.H. et al. (2016) SPATA2 links CYLD to LUBAC, activates CYLD, and controls LUBAC signaling. *Mol. Cell* **63**, 990–1005 <https://doi.org/10.1016/j.molcel.2016.08.001>
- 59 Iwai, K. (2021) LUBAC-mediated linear ubiquitination: a crucial regulator of immune signaling. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* **97**, 120–133 <https://doi.org/10.2183/pjab.97.007>
- 60 Zhu, J. and Tsai, N.P. (2020) Ubiquitination and E3 ubiquitin ligases in rare neurological diseases with comorbid epilepsy. *Neuroscience* **428**, 90–99 <https://doi.org/10.1016/j.neuroscience.2019.12.030>
- 61 Laforet, P., Malfatti, E. and Vissing, J. (2017) Update on new muscle glycogenosis. *Curr. Opin. Neurol.* **30**, 449–456 <https://doi.org/10.1097/WCO.0000000000000484>
- 62 Thomsen, C., Malfatti, E., Jovanovic, A., Roberts, M., Kalev, O., Lindberg, C. et al. (2021) Proteomic characterisation of polyglucosan bodies in skeletal muscle in RBCK1 deficiency. *Neuropathol. Appl. Neurobiol.* 1–13 <https://doi.org/10.1111/nan.12761>
- 63 Krenn, M., Salzer, E., Simonitsch-Klupp, I., Rath, J., Wagner, M., Haack, T.B. et al. (2018) Mutations outside the N-terminal part of RBCK1 may cause polyglucosan body myopathy with immunological dysfunction: expanding the genotype-phenotype spectrum. *J. Neurol.* **265**, 394–401 <https://doi.org/10.1007/s00415-017-8710-x>
- 64 MacDuff, D.A., Reese, T.A., Kimmey, J.M., Weiss, L.A., Song, C., Zhang, X. et al. (2015) Phenotypic complementation of genetic immunodeficiency by chronic herpesvirus infection. *eLife* **4**, e04494 <https://doi.org/10.7554/eLife.04494>
- 65 Tokunaga, F., Sakata, S., Saeki, Y., Satomi, Y., Kirisako, T., Kamei, K. et al. (2009) Involvement of linear polyubiquitylation of NEMO in NF-kappaB activation. *Nat. Cell Biol.* **11**, 123–132 <https://doi.org/10.1038/ncb1821>
- 66 Peltzer, N., Darding, M., Montinaro, A., Draber, P., Draberova, H., Kupka, S. et al. (2018) LUBAC is essential for embryogenesis by preventing cell death and enabling haematopoiesis. *Nature* **557**, 112–117 <https://doi.org/10.1038/s41586-018-0064-8>
- 67 Oda, H., Beck, D.B., Kuehn, H.S., Sampaio Moura, N., Hoffmann, P., Ibarra, M. et al. (2019) Second case of HOIP deficiency expands clinical features and defines inflammatory transcriptome regulated by LUBAC. *Front. Immunol.* **10**, 479 <https://doi.org/10.3389/fimmu.2019.00479>
- 68 Sasaki, Y., Sano, S., Nakahara, M., Murata, S., Kometsani, K., Aiba, Y. et al. (2013) Defective immune responses in mice lacking LUBAC-mediated linear ubiquitination in B cells. *EMBO J.* **32**, 2463–2476 <https://doi.org/10.1038/emboj.2013.184>
- 69 Sasaki, K., Himeno, A., Nakagawa, T., Sasaki, Y., Kiyonari, H. and Iwai, K. (2019) Modulation of autoimmune pathogenesis by T cell-triggered inflammatory cell death. *Nat. Commun.* **10**, 3878 <https://doi.org/10.1038/s41467-019-11858-7>

- 70 Peltzer, N., Rieser, E., Taraborrelli, L., Draber, P., Darding, M., Pernaute, B. et al. (2014) HOIP deficiency causes embryonic lethality by aberrant TNFR1-mediated endothelial cell death. *Cell Rep.* **9**, 153–165 <https://doi.org/10.1016/j.celrep.2014.08.066>
- 71 Lee, B., Shin, C., Shin, M., Choi, B., Yuan, C. and Cho, K.S. (2021) The linear ubiquitin E3 ligase-Relish pathway is involved in the regulation of proteostasis in *Drosophila* muscle during aging. *Biochem. Biophys. Res. Commun.* **550**, 184–190 <https://doi.org/10.1016/j.bbrc.2021.02.135>
- 72 Seymour, R.E., Hasham, M.G., Cox, G.A., Shultz, L.D., Hogenesch, H., Roopenian, D.C. et al. (2007) Spontaneous mutations in the mouse Sharpin gene result in multiorgan inflammation, immune system dysregulation and dermatitis. *Genes Immun.* **8**, 416–421 <https://doi.org/10.1038/sj.gene.6364403>
- 73 Hogenesch, H., Gijbels, M.J., Offerman, E., van Hooft, J., van Bekkum, D.W. and Zurcher, C. (1993) A spontaneous mutation characterized by chronic proliferative dermatitis in C57BL mice. *Am. J. Pathol.* **143**, 972–982 PMID: [8362989](https://pubmed.ncbi.nlm.nih.gov/8362989/)
- 74 Hogenesch, H., Janke, S., Boggess, D. and Sundberg, J.P. (1999) Absence of Peyer's patches and abnormal lymphoid architecture in chronic proliferative dermatitis (cpdm/cpdm) mice. *J. Immunol.* **162**, 3890–3896 PMID: [10201907](https://pubmed.ncbi.nlm.nih.gov/10201907/)
- 75 Strickson, S., Campbell, D.G., Emmerich, C.H., Knebel, A., Plater, L., Ritorito, M.S. et al. (2013) The anti-inflammatory drug BAY 11-7082 suppresses the MyD88-dependent signalling network by targeting the ubiquitin system. *Biochem. J.* **451**, 427–437 <https://doi.org/10.1042/BJ20121651>
- 76 Sakamoto, H., Egashira, S., Saito, N., Kirisako, T., Miller, S., Sasaki, Y. et al. (2015) Gliotoxin suppresses NF- κ B activation by selectively inhibiting linear ubiquitin chain assembly complex (LUBAC). *ACS Chem. Biol.* **10**, 675–681 <https://doi.org/10.1021/cb500653y>
- 77 De Cesare, V., Johnson, C., Barlow, V., Hastie, J., Knebel, A. and Trost, M. (2018) The MALDI-TOF E2/E3 ligase assay as universal tool for drug discovery in the ubiquitin pathway. *Cell Chem. Biol.* **25**, 1117–1127.e1114 <https://doi.org/10.1016/j.chembiol.2018.06.004>
- 78 Oikawa, D., Sato, Y., Ohtake, F., Komakura, K., Hanada, K., Sugawara, K. et al. (2020) Molecular bases for HOIPINs-mediated inhibition of LUBAC and innate immune responses. *Commun. Biol.* **3**, 163 <https://doi.org/10.1038/s42003-020-0882-8>
- 79 Katsuya, K., Oikawa, D., Iio, K., Obika, S., Hori, Y., Urashima, T. et al. (2019) Small-molecule inhibitors of linear ubiquitin chain assembly complex (LUBAC), HOIPINs, suppress NF- κ B signaling. *Biochem. Biophys. Res. Commun.* **509**, 700–706 <https://doi.org/10.1016/j.bbrc.2018.12.164>
- 80 Johansson, H., Isabella Tsai, Y.C., Fantom, K., Chung, C.W., Kumper, S., Martino, L. et al. (2019) Fragment-based covalent ligand screening enables rapid discovery of inhibitors for the RBR E3 ubiquitin ligase HOIP. *J. Am. Chem. Soc.* **141**, 2703–2712 <https://doi.org/10.1021/jacs.8b13193>
- 81 Aguilar-Alonso, F., Whiting, A.L., Kim, Y.J. and Bernal, F. (2018) Biophysical and biological evaluation of optimized stapled peptide inhibitors of the linear ubiquitin chain assembly complex (LUBAC). *Bioorg. Med. Chem.* **26**, 1179–1188 <https://doi.org/10.1016/j.bmc.2017.11.047>
- 82 Jo, T., Nishikori, M., Kogure, Y., Arima, H., Sasaki, K., Sasaki, Y. et al. (2020) LUBAC accelerates B-cell lymphomagenesis by conferring resistance to genotoxic stress on B cells. *Blood* **136**, 684–697 <https://doi.org/10.1182/blood.2019002654>
- 83 Bulaklak, K. and Gersbach, C.A. (2020) The once and future gene therapy. *Nat. Commun.* **11**, 5820 <https://doi.org/10.1038/s41467-020-19505-2>
- 84 Dunbar, C.E., High, K.A., Joung, J.K., Kohn, D.B., Ozawa, K. and Sadelain, M. (2018) Gene therapy comes of age. *Science* **359**, eaan4672 <https://doi.org/10.1126/science.aan4672>
- 85 Waldrop, M.A., Karingada, C., Storey, M.A., Powers, B., Iammarino, M.A., Miller, N.F. et al. (2020) Gene therapy for spinal muscular atrophy: safety and early outcomes. *Pediatrics* **146**, e20200729 <https://doi.org/10.1542/peds.2020-0729>
- 86 Iftikhar, M., Frey, J., Shohan, M.J., Malek, S. and Mousa, S.A. (2021) Current and emerging therapies for Duchenne muscular dystrophy and spinal muscular atrophy. *Pharmacol. Ther.* **220**, 107719 <https://doi.org/10.1016/j.pharmthera.2020.107719>
- 87 Varea, O., Duran, J., Aguilera, M., Prats, N. and Guinovart, J.J. (2021) Suppression of glycogen synthesis as a treatment for Lafora disease: establishing the window of opportunity. *Neurobiol. Dis.* **147**, 105173 <https://doi.org/10.1016/j.nbd.2020.105173>
- 88 Nitschke, S., Chown, E.E., Zhao, X., Gabrielian, S., Petkovic, S., Guisso, D.R. et al. (2021) An inducible glycogen synthase-1 knockout halts but does not reverse Lafora disease progression in mice. *J. Biol. Chem.* **296**, 100150 <https://doi.org/10.1074/jbc.RA120.015773>
- 89 Israelian, L., Nitschke, S., Wang, P., Zhao, X., Perri, A.M., Lee, J.P.Y. et al. (2021) Ppp1r3d deficiency preferentially inhibits neuronal and cardiac Lafora body formation in a mouse model of the fatal epilepsy Lafora disease. *J. Neurochem.* **157**, 1897–1910 <https://doi.org/10.1111/jnc.15176>
- 90 Chown, E.E., Wang, P., Zhao, X., Crowder, J.J., Strober, J.W., Sullivan, M.A. et al. (2020) GYS1 or PPP1R3C deficiency rescues murine adult polyglucosan body disease. *Ann. Clin. Transl. Neurol.* **7**, 2186–2198 <https://doi.org/10.1002/acn3.51211>
- 91 Gumusgoz, E., Guisso, D.R., Kasiri, S., Wu, J., Dear, M., Verhalen, B. et al. (2021) Targeting Gys1 with AAV-SaCas9 decreases pathogenic polyglucosan bodies and neuroinflammation in adult polyglucosan body and Lafora disease mouse models. *Neurotherapeutics* **18**, 1414–1425 <https://doi.org/10.1007/s13311-021-01040-7>
- 92 Ahonen, S., Nitschke, S., Grossman, T.R., Kordasiewicz, H., Wang, P., Zhao, X. et al. (2021) Gys1 antisense therapy rescues neuropathological bases of murine Lafora disease. *Brain* awab194. <https://doi.org/10.1093/brain/awab194>