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Lining up for quality control: linear ubiquitin and proteotoxicity

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The post-translational modification of proteins with linear ubiquitin (Ub) has previously been shown to be important for regulating inflammatory and immunological signaling in response to cellular pathogens. However, other functions of linear Ub are currently poorly defined. In this issue of *The EMBO Journal*, van Well and colleagues define a new role for linear Ub in regulating the intracellular stability and proteotoxicity of protein aggregates implicated in neurodegenerative diseases. This work provides new insights into the pathologic and potential therapeutic implications for linear Ub in the pathogenesis of these disorders.

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See also: **EM van Well *et al*** (May 2019)

Poly-ubiquitination is a post-translational protein modification involved in the regulation of multiple cellular functions including protein quality control, cellular signaling, and DNA repair. The capacity for poly-ubiquitin (Ub) to influence these diverse aspects of cell biology results from the ability for Ub to form distinct conjugates at the Ub N-terminal methionine (M1) or one of seven lysine residues within the Ub sequence. While many Ub ligases are involved in regulating formation of lysine Ub conjugates, M1 Ub conjugates result from the specific activity of a single Ub ligase complex called the linear Ub chain assembly complex (LUBAC) (Iwai *et al*, 2014; Spit *et al*, 2019). LUBAC is comprised of three subunits (the catalytic E3 ligase HOIP, HOIL-1L, and SHARPIN) and functions to add linear Ub chains comprised of M1 linkages onto substrate proteins. Interestingly, like the specificity afforded by LUBAC-dependent ubiquitination, the hydrolysis of M1 linkages within linear poly-Ub chains is also a highly selective process mediated by the activity of select de-ubiquitinating enzymes (DUBs) such as OTULIN. LUBAC-dependent linear ubiquitination has been implicated in the regulation of immunological and inflammatory signaling in response to cellular pathogens (Iwai *et al*, 2014; Spit *et al*, 2019). This activity is primarily mediated through linear ubiquitination of regulatory proteins involved in modulating the activity of stress-responsive transcription factors such as NFκB. However, few other functions of LUBAC-dependent linear ubiquitination have been identified to date.

In this issue of *The EMBO Journal*, van Well *et al* define a new role for LUBAC-dependent linear ubiquitination in regulating cellular quality control in response to toxic protein aggregates (van Well *et al*, 2019). Using cell culture models, they show that the LUBAC complex is recruited to cytosolic aggregates comprised of a mutant huntingtin (Htt) protein (Fig 1). This recruitment is mediated through direct interaction between the catalytic LUBAC subunit HOIP and the AAA^+ ATPase p97—a quality control protein that extracts substrates from large complexes or membranes to facilitate their proteasomal degradation (van den Boom & Meyer, 2018). Importantly, the p97-dependent recruitment of LUBAC to these aggregates increases linear ubiquitination of Htt, suggesting a functional role for this recruitment in regulating the stability and/or proteotoxicity of Htt aggregates.

Using a combination of genetic strategies, van Well *et al* show that increased LUBAC-dependent ubiquitination of Htt aggregates correlates with reduced levels of intracellular aggregates. This effect was not influenced by overexpression of an NFκB super-repressor, suggesting that reductions in aggregates occur independent of signaling through the NFκB pathway. Treating cells with proteasome inhibitors blocked reductions in Htt aggregates afforded by overexpression of the LUBAC catalytic subunit HOIP, suggesting that linear ubiquitination promotes aggregate clearance through increased targeting to proteasomal degradation. Intriguingly, van Well *et al* show that HOIP localization to Htt aggregates can reciprocally recruit p97 to these aggregates, suggesting a potential mechanism to increase p97-dependent extraction of Htt subunits for proteasomal degradation, although the specific involvement of p97 in this process remains to be tested. However, despite reducing aggregate levels, it remains unclear whether LUBAC directly promotes aggregate clearance and/or reduces aggregate formation, which is an interesting mechanistic question to be addressed in subsequent experimental efforts. Regardless, these results highlight a new role for LUBAC-dependent linear ubiquitination in regulating intracellular levels of toxic Htt aggregates implicated in the pathogenesis of the neurodegenerative disorder Huntington's disease (HD) (Jimenez-Sanchez *et al*, 2017).

Apart from reducing aggregate levels, van Well *et al* show that LUBAC-dependent linear ubiquitination remodels the surface of Htt aggregates to influence specific aspects of aggregate-associated proteotoxicity. Aberrant interactions between Htt aggregates and low complexity transcription factors such as SP1 are major contributors to aggregate

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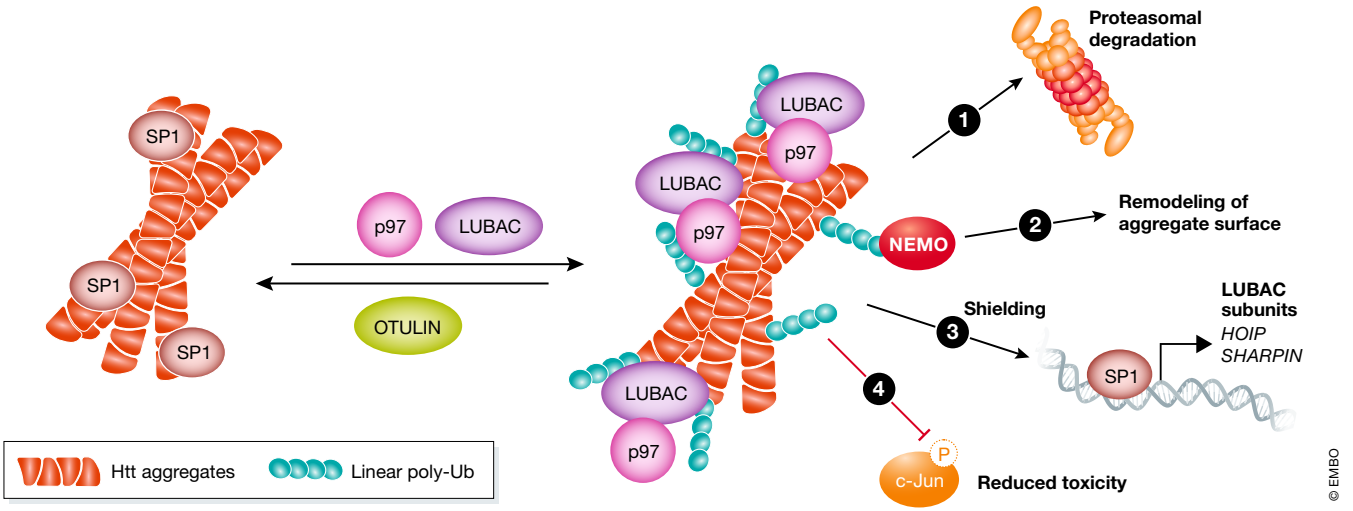

Figure 1. LUBAC-dependent regulation of Htt aggregates.
Illustration showing the molecular mechanisms involved in recruiting LUBAC to Htt aggregates and the functional implications of this activity on cellular function. LUBAC is recruited to Htt aggregates through a mechanism involving the AAA^+ ATPase p97. Once localized, LUBAC poly-ubiquitinates Htt aggregates to influence multiple aspects of aggregate biology. 1) LUBAC-dependent poly-ubiquitination reduces aggregate levels through a process likely involving increased targeting of Htt subunits to the proteasome. 2) Linear Ub chains on Htt aggregates can recruit proteins such as NEMO that bind to linear M1 Ub linkages to remodel the aggregate surface and potentially induce localized signaling. 3) LUBAC-dependent ubiquitination shields aggregates to prevent aberrant interactions with low complexity transcription factors such as SP1. Blocking SP1 interactions with aggregates increases SP1 transcriptional activity, which includes the regulation of LUBAC subunits such as *HOIP* and *SHARPIN*. 4) LUBAC-dependent linear ubiquitination of Htt aggregates suppresses phosphorylation of c-Jun, reflecting reduced signaling through the JNK/c-Jun signaling pathway.

proteotoxicity implicated in HD (Jimenez-Sanchez *et al*, 2017). The addition of linear poly-Ub to the aggregate surface could function as a "shield" to prevent these aberrant interactions and restore transcription factor activity. Consistent with this, van Well *et al* show that LUBAC-dependent ubiquitination correlates with reductions in the localization of SP1 to Htt aggregates and increases SP1 transcriptional activity. Interestingly, they go on to demonstrate that SP1 transcriptionally regulates the expression of LUBAC subunits, suggesting that reducing association of SP1 with Htt aggregates could transcriptionally stimulate LUBAC activity through a positive feedback signaling loop. Collectively, these results indicate that linear ubiquitination can serve as a protective mechanism to prevent aberrant interactions between Htt aggregates and transcription factors involved in HD-associated proteotoxicity.

Apart from shielding aggregates, remodeling the aggregate surface through linear ubiquitination could also provide a platform to recruit signaling factors. Numerous proteins including the NFκB regulator NEMO contain domains that bind to linear Ub chains (Iwai *et al*, 2014; Spit *et al*, 2019). Interestingly, van Well *et al* show that NEMO localizes to Htt aggregates through a process dependent on LUBAC activity, suggesting that LUBAC-dependent ubiquitination recruits NEMO to the aggregate surface. While the functional implications of the recruitment of NEMO (or other linear Ub binding proteins) to Htt aggregates remain to be defined, this does suggest that remodeling the aggregate surface could provide a potential platform to regulate localized signaling that could be important for adapting cellular physiology in response to toxic Htt aggregation.

The ability for LUBAC-dependent linear ubiquitination to reduce aggregate levels and reduce aberrant interactions with transcription factors suggests that this process should reduce proteotoxicity associated with Htt aggregation. Consistent with this, van Well *et al* show that genetically increasing LUBAC activity in cell culture models or primary cells reduces signaling through the JNK/c-Jun signaling pathway (Fig 1). In contrast, reducing linear ubiquitination by overexpressing *OTULIN* increases JNK/c-Jun signaling. Importantly, the relationship between linear ubiquitination of Htt aggregates and JNK/c-Jun signaling is independent of NFκB activity, further supporting a model whereby LUBAC activity reduces proteotoxicity-associated signaling through the direct regulation of Htt aggregate quality control.

The above results highlight a new role for LUBAC-dependent linear ubiquitination in regulating intracellular stability and proteotoxicity of Htt aggregates. However, a key question relates to the importance of this process in the Htt aggregate toxicity associated with the pathogenesis of HD. To begin to address this question, the authors show increased localization of LUBAC subunits and linear Ub chains on Htt aggregates in the striatum and frontal cortex of HD mouse models and in cortical sections from HD patients. This indicates that LUBAC-dependent linear ubiquitination of Htt aggregates also occurs during the progression of HD *in vivo*. Furthermore, van Well *et al* show that the expression of LUBAC subunits is significantly reduced in mouse models of HD and in HD patients, suggesting that dysregulation of LUBAC-dependent linear ubiquitination is a potential contributing factor in the pathogenesis of this disease. While the functional importance for linear ubiquitination of Htt aggregates *in vivo* remains to be further established, the results described in this manuscript provide a new framework to address the functional importance for this process in HD progression.

The discovery that LUBAC-dependent ubiquitination has a key role in regulating

cellular quality control opens many new lines of scientific inquiry to define the implications of this process both in HD and in other protein aggregation diseases. One key question relates to the potential for LUBAC-dependent ubiquitination to influence the stability and proteotoxicity of other types of protein aggregates. Intriguingly, van Well *et al* show that LUBAC-dependent ubiquitination reduces intracellular aggregates comprised of the ALS-associated protein TDP-43 through a proteasome-dependent mechanism similar to that observed for Htt aggregates. While this suggests that linear ubiquitination could represent a general cellular quality control mechanism for protein aggregates, it will be interesting to continuing defining how this process impacts aggregate stability and proteotoxicity in the context of other types of protein aggregation diseases. Further, considering the specificity for LUBAC and OTULIN in regulating linear poly-Ub chains, the results described in this manuscript suggest new therapeutic opportunities to influence cellular quality in the context of protein aggregation disorders through the selective targeting of these two enzymes. As further studies are directed to define the importance of LUBAC-dependent regulation of quality control in neurodegenerative disease progression, the potential for selectively targeting the regulation of linear poly-Ub to mitigate aggregate proteotoxicity without globally disrupting inflammatory and immunological signaling will be revealed.

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