PhotoDisc/ Getty Images

Lys33-linked ubiquitin in post-Golgi transport

> “Lys33-linked polyubiquitylation targets CRN7 to theTGN, where it stabilizes F-actin to facilitate post-Golgi transport”

Polyubiquitylation is a versatile and reversible post-translational modification that regulates protein function. Ubiquitin molecules can be assembled into chains of varying length and linkages by using one of seven ubiquitin Lys residues or the Met1 residue. These structurally distinct chains have different cellular functions; however, the physiological roles of some types of ubiquitin chains, including atypical chains linked by Lys33, are poorly understood. Yuan *et al*. now report that Lys33-linked polyubiquitylation of the F-actin regulator coronin 7 (CRN7) promotes post-Golgi transport.

The authors found that Kelch-like protein 20 (KLHL20), a substrate adaptor of Cullin 3 (CUL3)-based E3 ubiquitin ligases, localized at the *trans*-Golgi network (TGN). KLHL20 localization at the TGN was dependent on ADP-ribosylation factor (ARF) GTPases, which control Golgi-mediated vesicular trafficking, and they therefore investigated a possible role of KLHL20 in protein trafficking. Indeed, anterograde transport of GFP–VSVG (vesicular stomatitis virus glycoprotein) and GFP-tagged MPR (mannose-6-phosphate receptor) from the TGN to the plasma membrane and to endosomes, respectively, was blocked in KLHL20-depleted cells. Conversely, retrograde transport of cargo from the plasma membrane to the endoplasmic reticulum through the Golgi was unaffected. Moreover, the authors found that KLHL20-mediated anterograde trafficking was dependent on KLHL20 binding to CUL3, which indicates that the CUL3–KLHL20 E3 ubiquitin ligase complex has a role in post-Golgi protein trafficking.

Cargo exit from the TGN requires the formation of tubular carrier precursors, which then undergo scission to produce post-Golgi carriers. In *KLHL20*-knockdown cells, the formation and elongation of such tubular carriers was impaired, which suggests that the CUL3–KLHL20 E3 ubiquitin ligase functions in these processes. To further elucidate the mechanisms by which CUL3–KLHL20 regulates post-Golgi transport, the authors performed a two-hybrid screen. Among the KLHL20 substrates identified in this screen was CRN7, which is known to be crucial for the post-Golgi transport of VSVG and MPR. KLHL20 and CRN7 directly interacted, and CUL3–KLHL20 promoted CRN7 polyubiquitylation. Interestingly, ubiquitin mutants, in which either each Lys residue was substituted or in which all Lys residues but one Lys residue were substituted, showed that CUL3–KLHL20 generates Lys33-linked chains on CRN7. Furthermore, Lys33-linked CRN7 polyubiquitylation was required for VSVG and MPR post-Golgi transport.

How is trafficking affected by CUL3–KLHL20-mediated CRN7 polyubiquitylation? The formation of TGN tubular carrier precursors is actin-dependent, and the authors found that Lys33-linked CRN7 polyubiquitylation contributed to F-actin assembly at the TGN. Importantly, they found that CRN7 that was modified by a Lys33-linked chain *in vivo* specifically bound to the ubiquitin-binding adaptor protein epidermal growth factor receptor substrate 15 (EPS15), and that this promoted CRN7 recruitment to the TGN.

Thus, CUL3–KLHL20-dependent Lys33-linked polyubiquitylation targets CRN7 to the TGN, where it stabilizes F-actin to facilitate post-Golgi transport. These findings provide evidence for a positive signalling (and non-proteolytic) role of this atypical ubiquitin chain and a mechanism for its recognition.

Kim Baumann

ORIGINAL RESEARCH PAPER Yuan, W.-C. *et al.* K33-linked polyubiquitination of coronin 7 by Cul3–KLHL20 ubiquitin E3 ligase regulates protein trafficking. *Mol. Cell* http://dx.doi.org/10.1016/j.molcel.2014.03.035 (2014)