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Previews

Linking ubiquitin to actin dynamics during cell fusion

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Cell-cell fusion is essential to the development of multicellular organisms and is driven by remodeling of the actin cytoskeleton. In this issue of *Developmental Cell*, Rodríguez-Pérez et al. reveal how CRL3-dependent mono-ubiquitylation modulates cell fusion by controlling the dynamics of cytoskeletal rearrangements.

Cullin-RING ubiquitin ligases (CRLs) are multi-subunit complexes that mediate the ubiquitylation of a large number of substrates. The latter are recognized via the exchangeable substrate receptor (SR) subunits of the CRLs. Mammals have eight cullin proteins, each of which selectively interacts with a subset of SRs, enabling the assembly of ~230 distinct CRL-SR complexes (Skaar et al., 2013). Unlike other well-characterized CRLs, which seem to solely regulate proteolytic ubiquitylation, CRL3s regulate both proteolytic ubiquitylation (by mediating the formation K48-linked ubiquitin chains) and non-proteolytic ubiquitin signals (via mono-ubiquitylation and K33- and K63-linked ubiquitin chains) (Jerabkova and Sumara, 2019). In this issue of *Developmental Cell*, Rodríguez-Pérez et al. (2021) show that $CRL3^{KCTD10}$ is a critical regulator of cell-cell fusion via EPS8 mono-ubiquitylation, suggesting new, unexplored functions of CRL3-mediated non-proteolytic ubiquitylation.

Cell-cell fusion plays an essential role in the development of multicellular organisms by regulating processes such as fertilization and the formation of placenta and skeletal muscle (Lee and Chen, 2019). To perform fusion, cells dynamically remodel their plasma membranes by restructuring the actin cytoskeleton, which, in turn, controls the formation of invasive protrusions (i.e., filopodia) and finalizes the fusion process (Segal et al., 2016). The actin remodeler EPS8 binds plasma membranes via a split pleckstrin homology domain and plays a central role in filopodia formation. Specifically, EPS8 promotes the crosslinking of actin filaments when in complex with IRSp53 (insulin receptor tyrosine kinase substrate of 53 KD) (Vaggi et al., 2011). While the role of actin filament assembly in cell membrane fusion has long been studied, there is comparatively little knowledge about the molecular mechanisms underlying the depolymerization of actin filaments, which allows for a dynamic modulation of cell membrane fusion.

Rodríguez-Pérez et al. (2021) focused on myogenic cell fusion, a fundamental process involving the attachment of myoblasts to receiving myotubes to form skeletal muscle (Lee and Chen, 2019). The authors analyzed the impact of all SRs of CRL2 and CRL3 complexes on cell membrane fusion and found that KCTD10, a CRL3 SR, is required for myoblast fusion. Next, in an elegant series of studies, the authors investigated whether KCTD10 orchestrates cell fusion by regulating the ubiquitylation of specific cellular substrates. Using mass spectrometry, the authors analyzed the KCTD10 interactome and detected binding of KCTD10 to several actin regulators, including EPS8 and IRSp53. The authors carefully dissected the molecular mechanisms underlying the KCTD10-mediated regulation of EPS8 and IRSp53. They discovered that $CRL3^{KCTD10}$ is recruited to the plasma membrane at the regions of cell-cell contact to promote EPS8 mono-ubiquitylation, which results in the displacement of the EPS8-IRSp53 complex from the membrane at cell-cell interfaces (Figure 1). As part of this membrane-disengagement mechanism, the authors discovered that KCTD10 interacts with EPS8 and IRSp53 through their respective membrane-recruitment domain, suggesting that KCTD10 may also promote EPS8-IRSp53 delocalization by competing for their binding to membrane structural elements.

These results prompted the authors to test whether successful cell fusion is dependent on cycles of recruitment and displacement of EPS8-IRSp53 at sites of cell contact. They found that KCTD10-depleted cells accumulate actin bundles at regions of cell contact and are unable to perform fusion, despite the presence of protrusions projecting from the myoblasts to the receiving myotube. Taken together, these experiments reveal that $CRL3^{KCTD10}$ limits formation of cortical actin bundles to allow successful cell fusion upon membranes pairing. Indeed, while transient recruitment of EPS8 to sites of cell contact promotes cell fusion, constitutive localization of EPS8 to plasma membranes inhibits this process. Therefore, EPS8-IRSp53 removal is necessary to terminate the process of actin bundling and allow pairing cells to progress with cell membrane fusion (Figure 1).

Of note, the authors discovered that $CRL3^{KCTD10}$ not only displaces EPS8-IRSp53 from sites of cell contact, but also represses its actin bundling activity. This dual function of KCTD10 synchronizes EPS8-IRSp53 displacement from the sites of cell contact with inhibition of actin bundling, and may prevent unscheduled and detrimental cytoskeletal rearrangements in cellular compartments distal from the membrane fusion sites.

Plasma membrane fusion is an essential and ubiquitous phenomenon in

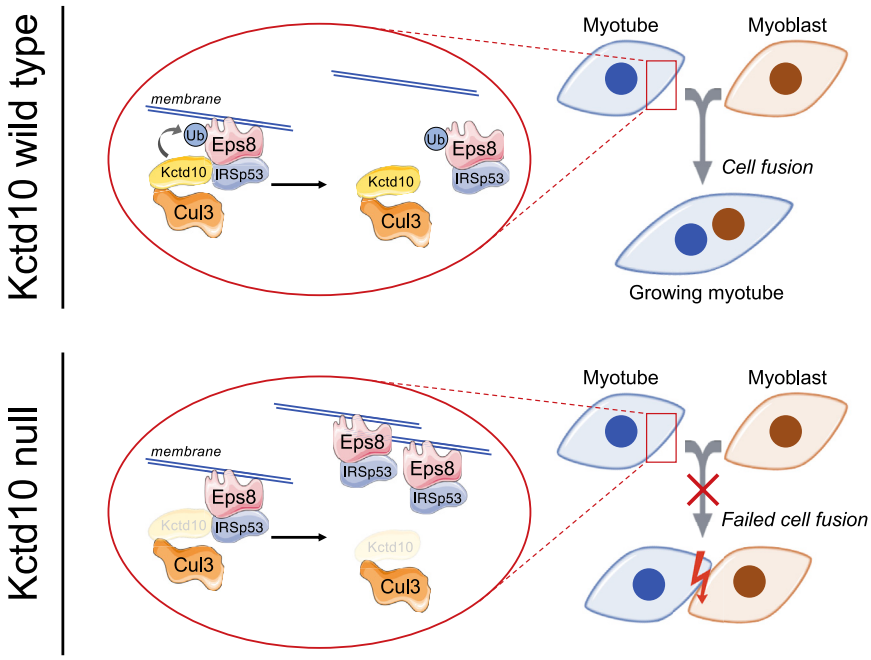

Figure 1. Ubiquitin-mediated regulation of myotube fusion
Top: CRL3^{KCTD10}-mediated mono-ubiquitylation of ESP8 triggers EPS8-IRSp53 complex displacement from the plasma membrane of the receiving myotube, allowing the cells to successfully perform cell fusion. Bottom: KCTD10-depletion in the receiving myotube prevents ESP8 ubiquitylation, inducing accumulation of the EPS8-IRSp53 complex at cell membranes. In turn, increased levels of this complex at cell membranes impede the completion of cell fusion.

living organisms, yet the molecular mechanisms regulating cell-cell fusion remain to be defined in more detail. Importantly, defective cell fusion underlies the pathogenesis of several diseases, including muscle and skeletal disorders (Lee and Chen, 2019). The work by Rodríguez-Pérez et al. (2021) provides a mechanistic link between the ubiquitin system and cell fusion and defines a conserved pathway that leverages mono-ubiquitylation to orchestrate the remodeling of the actin cytoskeleton. Complementing the myogenic role that the authors observed within the muscle precursor cells, recent studies have revealed that mutations in several CRL3 SRs are associated with skeletal muscle disorders (Gupta and Beggs, 2014).

Well-characterized CRL1s, CRL2s, and CRL4s catalyze the formation of K48-linked ubiquitin chains, which target modified substrates for proteasomal degradation. In contrast, CRL3s also have the ability to promote mono-ubiquitylation and the formation of different ubiquitin chains (Jerabkova and Sumara, 2019). The molecular mechanisms and the evolutionary reasons for this polyhedric and unique function of CRL3 complexes are not understood. What is clear is that the irreversibility of the ubiquitin-proteasome system (UPS) makes it a perfect way to regulate processes that must proceed unidirectionally (e.g., cell cycle and circadian oscillations) (Skaar et al., 2013). In contrast, this same intrinsic feature makes the UPS unsuitable for biological events that require a transient and bidirectional regulation. Thus, the evolutionary choice to employ non-proteolytic mono-ubiquitylation for the modulation of cell fusion better suits the necessity of achieving a dynamic and reversible regulation of this process. In this context, it would be interesting to understand whether the CRL1^{FBXW5}-mediated degradation of ESP8 also plays a role in regulating cell fusion (Werner et al., 2013).

Looking forward, these studies open several research avenues, including the investigation of the molecular mechanisms that allow CRL3 complexes to use different ubiquitin codes to regulate cellular functions. Also relevant will be the identification of deubiquitylating enzymes that may dynamically regulate EPS8 and other factors in response to cell fusion cues (Clague et al., 2019) and the discovery of ubiquitin binding domain-containing proteins that may be recruited to modulate the formation of specific signaling complexes controlling actin remodeling (Dikic et al., 2009). Overall, the results of this work contribute to a deeper understanding of the fundamental mechanisms regulating cell fusion and will potentially provide new tools to harness the ubiquitin system for the therapy of diseases driven by defective cell fusion.

DECLARATION OF INTERESTS

M.P. is a consultant for, is on the SAB of, and has financial interests in Coho Therapeutics, CullGen, Kymera Therapeutics, Santi Therapeutics, and SEED Therapeutics. He is also a cofounder of Coho Therapeutics. L.L. declares no competing interests.

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