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Parallel Parkin: Cdc20 Takes a New Partner

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Abstract

CDC20 and CDH1 are well-established substrate receptors for the Anaphase Promoting Complex/Cyclo-some (APC/C). In this issue of *Molecular Cell,* Lee et al. (2015) show that these adaptors can also target cell cycle proteins for destruction through a second ubiquitin ligase, Parkin.

The APC/C is the largest and most complex ubiquitin ligase thus far characterized, with 19 subunits totaling 1.5 mega-daltons. The core APC/C interacts with either of two proteins, CDC20 or CDH1, which bind substrates and also activate the APC/C catalytically. It has often been assumed that the complexity of the APC/C is required for its critical role in timing anaphase onset, which is initiated by the degradation of APC/C substrates and takes place in an all-or-none fashion only after all chromosomes have been attached to the spindle. This idea is belied, however, by the finding of Lee et al. that Parkin, a monomeric ubiquitin ligase, is able to substitute, at least in part, for the entire APC/C by binding CDC20 or CDH1 and ubiquitinating "APC/C substrates" (Lee et al., 2015). This novel mitotic function for Parkin resolves previously unexplained observations that loss of Parkin causes errors in chromosome segregation, which could not be explained by Parkin's established role in mitophagy and connection with Parkinson's disease.

CDC20/CDH1 binding to Parkin and the APC/C appears to be mutually exclusive (Lee et al., 2015), raising questions about how Parkin uses these substrate receptors to bind and ubiquitinate APC/C targets. CDC20 and CDH1 recognize several degrons, the best characterized of which is the D-box. In vitro ubiquitination of Cyclin B and securin by the Parkin^{CDC20} complex requires the D-box of these substrates. In the APC/C, however, CDC20/ CDH1 are actually D-box co-receptors, acting with the core APC/C subunit APC10, such that the D-box is sandwiched between CDC20/CDH1 and the neighboring APC10 subunit (da Fonseca et al., 2011). It is possible that the receptors work independently in the context of Parkin: APC10 is not essential for APC/ C$^{CDC20/CDH1}$ function in yeast (Carroll and Morgan, 2002), and so CDC20/ CDH1 alone might provide sufficient substrate affinity to allow efficient ubiquitination by Parkin. Alternatively, some other domain of Parkin could provide this co-receptor role, or Parkin could interact with other degron motifs on the substrates. Resolution of this question will likely have to await a structural study of the Parkin receptor complex with substrate.

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The APC/C is regulated in several ways to promote initiation of anaphase at the right time, but it remains unclear whether these mechanisms also control Parkin$^{CDC20/CDH1}$ activity (see Figure 1). Emi1 and Mad2 are essential APC/C inhibitors. Mad2 inhibits CDC20 (Fang et al., 1998), and it might therefore block Parkin^{CDC20} activity or assembly. Emi1 has several mechanisms of action. It acts as a D-box pseudosubstrate (Miller et al., 2006), which could also target the Parkin^{CDH1} complex. However, Emi1 also appears to antagonize APC/C-spe-cific E2 ubiquitin-conjugating enzymes (Wang and Kirschner, 2013). Interestingly, Lee et al. find that Plk1, an important regulator of the APC/C, also regulates the mitotic activity of Parkin. They demonstrate that Plk1 phosphorylates Parkin at Ser378, a residue within the RBR (RING-between-RING) domain, during mitosis, and they show that this modification is essential for Parkin-CDC20 binding, enhances the ubiquitination activity of Parkin, and consequently promotes turnover of key mitotic regulators. Parkin's mitotic function may also be regulated by Parkin degradation, as Lee et al. also show that Parkin protein levels change during the cell cycle, peaking from G2 to early G1. Further experiments are necessary to determine what mediates Parkin degradation, but it likely involves phosphorylation-mediated ubiquitination. The mitotic activity of Parkin is regulated by localization as well. Parkin co-localizes with Plk1 at the centrosomes in early mitosis and migrates to the midbody during telophase (Lee et al., 2015). At present, it is not clear what controls Parkin localization or how localization affects its activity.

The human APC/C binds two E2s, Ube2C and Ube2S. Ube2C first monoubi-quitinates lysines on the substrate and subsequently utilizes one of the seven lysines on ubiquitin to generate short ubiq-uitin chains (Meyer and Rape, 2014). Ube2S, the second APC/C cognate E2, then adds Lys11-linked ubiquitin chains, resulting in the formation of branched pol-yubiquitin chains (Meyer and Rape, 2014). In contrast, Parkin, a RBR E3 ubiquitin ligase, accepts ubiquitin from the E2 enzyme onto an active site cysteine, and then Parkin itself transfers ubiquitin onto substrates, thereby determining the ubiq-uitin linkage type (Wenzel et al., 2011). Lee et al., 2015 show that, much like the mammalian APC/C, Parkin appears to generate Lys11-linked chains on its mitotic substrates, although in vitro experiments are needed to confirm these initial findings. Interestingly, Parkin has previously been shown to generate Lys6-, Lys11-, Lys48-, and Lys63-linked chains in its well-known role in mitophagy (Durcan and Fon, 2015). In mitophagy, Parkin is activated by PINK1-mediated phosphorylation of its UBL domain at Ser65 and is allosterically activated by binding PINK1-phosphorylated ubiquitin (Durcan and Fon, 2015). Perhaps binding to CDC20 and CDH1, or phosphorylation by Plk1, causes Parkin to favor the synthesis of Lys11-linked chains during mitosis, whereas binding to phosphory-lated ubiquitin or Parkin phosphorylation by PINK1 may promote the synthesis of other linkage types during mitophagy. Future studies will be required to elucidate the mechanism by which Parkin is capable of synthesizing distinct ubiquitin linkage types in these different contexts.

The APC/C is thought to be the main regulatory node controlling chromosome segregation, but the work by Lee et al. suggests that Parkin also regulates mitosis by using CDC20 and CDH1 to bind and ubiquitinate mitotic regulators. However, it is important to note that this is an unequal partnership, since the APC/C is essential, while Parkin is not essential and is absent in many organisms that have the APC/C. Therefore, the APC/ C and Parkin likely have distinct mitotic functions. It is interesting to note that Parkin has previously been

shown to function with a different Cullin-RING ligase in neurons (Staropoli et al., 2003). Perhaps Parkin is specialized for forming a particular type of ubiquitin chain on mitotic substrates or better targets specific substrates in particular subcellular contexts. During mitophagy, Parkin may be limiting and unavailable for its mitotic functions, providing some form of crosstalk between these pathways. Future studies will be necessary to determine the precise role of Parkin-mediated ubiquitination in mitosis.

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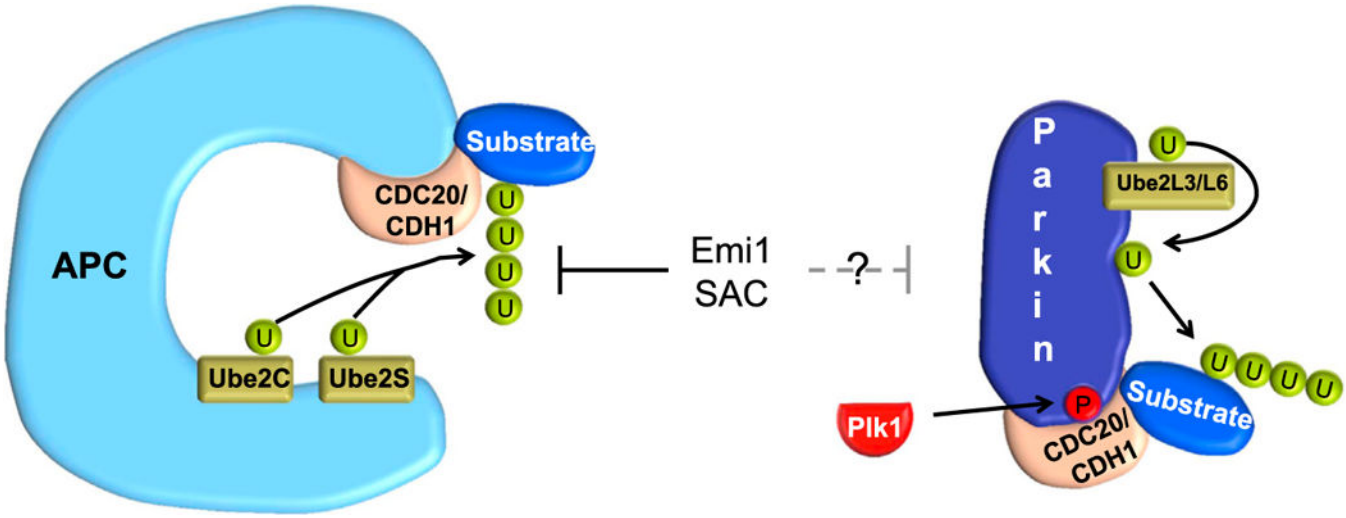

Figure 1. The APC and Parkin Bind and Ubiquitinate Protein Targets through the Substrate Adaptors CDC20 and CDH1

The spindle assembly checkpoint (SAC) and Emil regulate the activity of the APC^{CDC20} and APC^{CDH1} complexes. The SAC and Emil may also regulate the $Parkin^{CDC20/CDH1}$ complexes. The binding of both CDC20 and CDH1 to Parkin is promoted by phosphorylation of Parkin at Ser378 by Plkl.