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Cdc20, an activator at last

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Summary

In this issue of Molecular Cell, Kimata et al. (2008) show that Cdc20 functions not only in the recruitment of substrates to the Anaphase Promoting Complex, but also in its activation.

Cdc20 was first identified as an essential gene required for the metaphase to anaphase transition in yeast. Subsequent work suggested that the *Drosophila* Cdc20 homologue, Fizzy, was required for the turnover of mitotic cyclins (reviewed in Thornton and Toczyski 2006). It soon became apparent that Cdc20/Fizzy and a similar protein, Cdh1/Fizzy-Related, promoted Anaphase Promoting Complex (APC) function, although the mechanism by which it did this was not clear. What was clear early on, however, was that the regulation of APC activity on its substrates relied largely on the regulation of Cdc20 and Cdh1. Thus, not only were these molecules required for APC function, they were also the targets of its regulation, either by their degradation, phosphorylation, or the binding of protein inhibitors. Now, Kimata et al (2008) have exploited an unusual APC substrate, NEK2A, which they had previously shown interacts directly with the core APC independently of Cdc20, to identify a previously unappreciated role for Cdc20 in direct APC activation.

Proof for a direct role for Cdc20 and Cdh1 in APC function came with *in vitro* ubiquitination assays. Although Cdc20 and Cdh1 were originally referred to as APC *activators*, it soon became apparent that an important aspect of their function was to recognize APC substrates and promote their recruitment to the APC. This was based largely on the fact that Cdc20 and Cdh1 bound directly to APC substrates and that this binding depended, in most cases, upon the destruction motifs present in the substrates. APC substrates are recognized by the presence of one or more copies of any of several loosely defined sequences: the best characterized being the D (Destruction) box and the KEN box. Recognition of these motifs by Cdc20 and Cdh1 is thought to occur through a WD40 domain, which is found in each of these molecules. Thus, although these molecules were initially thought of as APC *activators*, the model that quickly emerged suggested that they function primarily as substrate *adaptors*. This model was biased in part by similarities between the APC and a related RING finger ubiquitin ligase, the SCF. Like the APC, the SCF uses a set of associated adaptors, called F-box proteins, that recognize specific substrates (Thornton and Toczyski 2006). Moreover, many F-box proteins also use WD40 domains for the purpose of substrate recognition. Biochemical studies of the SCF, which is better understood than the APC mechanistically, suggest that F-box proteins are unlikely to directly activate the SCF, and this has helped direct the field toward a view in which the APC adaptors do not directly activate the catalytic activity of the APC.

Although there have been some hints that subunits of the core APC can recognize substrates directly through their destruction motifs (Passmore, McCormack et al. 2003; Yamano, Gannon et al. 2004; Carroll and Morgan 2002), in almost all cases it is clear that either the Cdc20 or Cdh1 adaptor is also required for substrate binding. In these cases, it was not possible to dissociate the roles of these adaptors in recruiting substrates from any other roles they might have. The one well-characterized exception to the accepted view of how the APC binds substrates is provided by the NIMA-related kinase Nek2A. Nek2A associates directly with the

core APC through a C-terminal MR (for methionine-arginine) motif (Hayes, Kimata et al. 2006). Interestingly, this mode of association might be similar to the way in which the adaptors themselves associate with the APC. Cdc20 and Cdh1 have three well-characterized motifs. Most of the C terminus consists of the aforementioned WD40 motif. In addition, these proteins encode two highly conserved motifs important for their association with the APC: the “C-box” and the “IR tail”. The C-box is located in the N terminus, and is essential for the ability of adaptors to bind the APC and promote ubiquitination *in vivo* and *in vitro*. In addition, the two terminal residues of the adaptors are almost without exception an isoleucine followed by an arginine (IR). This tail promotes the association of the adaptors with the TPR (tetratricopeptide repeat) subunits of the APC. Although Nek2A can bind the core APC directly through its MR tail, presumably in a manner analogous to the means by which the Cdc20 and Cdh1 IR tails bind the core APC, Cdc20 is still required for Nek2A turnover (Hayes, Kimata et al. 2006).

Kimata et al. (2008) have now elegantly explained the requirement for Cdc20 in Nek2A turnover by showing that Cdc20 can activate the APC directly. Cdc20 promotes the activity of the APC toward Nek2A *in vitro*. This activation is clearly distinct from its previously described function, as it does not require the C-terminal half of Cdc20, which contains the WD40 and IR motifs. This fits nicely into their model: the role of the WD40 domain in substrate recruitment is not required in this particular case, as Nek2A can bind the APC directly. Interestingly, the Cdc20 IR motif is not required for Cdc20 function in yeast; rather it contributes to APC-dependent Cdc20 turnover (Thornton, Ng et al. 2006), similar to the function of the Nek2A MR motif in its turnover. APC-catalyzed Nek2A ubiquitination does require the Cdc20 C-box. It is not yet clear whether C-box binding directly activates the APC, or if other Cdc20 domains are involved. While a precise understanding of how Cdc20 mediates APC activation will probably await an APC crystal structure, the EM structure of the APC with and without its associated adaptor suggests that the binding of the adaptor molecule promotes higher order change in the APC (Dube, Herzog et al. 2005).

Interestingly, the authors note that similar C-box-like (CL) motifs exist in other putative ubiquitin ligases, and in the non-essential APC subunit Doc1/Apc10. Like Cdc20, Doc1 participates in substrate binding, harbors a C-terminal LR motif (similar to the IR in Cdc20), and stimulates APC activity. By contributing to the affinity of the APC for substrates, Doc1 promotes processivity of the ubiquitination reaction (Carroll and Morgan 2002). The Doc1 crystal structure shows that the CL motif lies on the ligand-binding interface (Wendt, Vodermaier et al. 2001; Au, Leng et al. 2002), and substitutions in an adjacent β strand affect its processivity function (Carroll, Enquist-Newman et al. 2005). In the future it will be interesting to test whether directed mutations within the CL motif affect Doc1 function. These studies could lend support to the idea that C-box and CL motifs in diverse ubiquitin ligases have a conserved role in the activation of ubiquitination reactions.

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