dimer interface as eIF2α, related by crystal packing, it is still not in an active conformation. It is not autophosphorylated and in the absence of phosphorylation of the activation loop, the conserved Glu643 residue of the αC-helix actually interacts with the HRD Arg834. GCN2 thus remains in an inactive conformation. Clearly dimerization is not sufficient to promote autophosphorylation or to snap the αC-helix into its active conformational state. GCN2, but not PERK and HRI, lacks both basic residues in the αC-helix, and this may explain why activation is not achieved so readily by simple dimerization. Although the precise mechanism remains to be elucidated, dimerization and phosphorylation of the activation loop appear to be closely linked for PKR, with dimerization being an essential first step.

Binding of a signal molecule to the N-terminal regulatory domain promotes dimerization and activation of each eIF2α kinase. PKR is activated by the binding of dsRNA to the N-terminal regulatory domain. In both the PKR and the GCN2 structures, the regulatory domain has been deleted. Therefore, how binding of dsRNA promotes PKR dimerization and activation cannot be resolved and is largely overlooked in these papers. In addition, the eIF2α kinases have a long β4-β5 linker. In both cases, this segment was truncated and what remains is disordered. Both regions could contribute directly to the activation mechanism; without the full-length structures it is premature to speculate on the ordered pathway for activation. Is the activation loop locked into an inhibited conformation in the absence of dsRNA in a way that involves the regulatory N-terminal domain or the β4-β5 linker? Does binding of dsRNA then release the inhibition? From the structure and mutagenesis studies it is clear that the allosteric mechanism for activation of the eIF2α kinases involves a tight and dynamic interaction between dimerization, autophosphorylation, and substrate docking. These studies reveal the intricacy of this integration and also demonstrate how phosphorylation of the activation loop can allosterically couple two remote sites.

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Selected Reading

Dar, A.C., Dever, T.E., and Sicheri, F. (2005). Cell *122*, this issue, 887–900.

Dey, M., Cao, C., Dar, A.C., Tamura, T., Ozato, K., Sicheri, F., and Dever, T.E. (2005). Cell *122*, this issue, 901–913.

Johnson, L.N., Noble, M.E., and Owen, D.J. (1996). Active and inactive protein kinases: structural basis for regulation. Cell *85*, 149–158.

Kim, C., Xuong, N.H., and Taylor, S.S. (2005). Science *307*, 690–696.

Lei, M., Lu, W., Meng, W., Parrini, M.C., Eck, M.J., Mayer, B.J., and Harrison, S.C. (2000). Cell *102*, 387–397.

Nolan, B., Taylor, S., and Ghosh, G. (2004). Mol. Cell *15*, 1–20.

Padyana, A.K., Qiu, H., Roll-Mecak, A., Hinnebusch, A.G., and Burley, S.K. (2005). J. Biol. Chem. *280*, 29289–29299.

Song, H., Hanlon, N., Brown, N.R., Noble, M.E., Johnson, L.N., and Barford, D. (2001). Mol. Cell *7*, 615–626.

Yang, J., Garrod, S.M., Deal, M.S., Anand, G.A., Woods, V.L., and Taylor, S.S. (2005). J. Mol. Biol. *346*, 191–201.

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CDKs Give Cdc6 a License to Drive into S Phase

The accumulation of Cdc6 promotes the initiation of DNA replication. In this issue of *Cell*, Mailand and Diffley (2005) show that phosphorylation of Cdc6 by cyclin-dependent kinases prevents its destruction by the anaphase promoting complex (APC). This simple mechanism explains how the APC simultaneously spares Cdc6 while targeting for destruction suppressors of DNA replication during the transition from quiescence to cell cycle reentry.

Eukaryotic cells have evolved extensive mechanisms to ensure that DNA replication origins fire only once per cell cycle. DNA replication is initiated by the assembly of prereplication complexes in a process known as licensing (Diffley, 2004). During quiescence, when cells have exited the cell cycle, the assembly of the prereplication complex is inhibited (Diffley, 2004; Blow and Dutta, 2005). One of the crucial regulatory pathways allowing cell cycle reentry following quiescence is the timely and ordered destruction of prereplication-complex inhibitors by the anaphase promoting complex (APC) ubiquitin ligase (Diffley, 2004).

There are two forms of the APC: the mitotic form, APC^{Cdc20}, containing the substrate-targeting subunit Cdc20, and the nonmitotic form, APC^{Cdh1}, containing the Cdc20 homolog Cdh1 (Harper et al., 2002). During the G1 phase of the cell cycle, the nonmitotic form of APC, APC^{Cdh1}, ensures prereplication-complex formation by degrading the inhibitors geminin and cyclin A (McGarry and Kirschner, 1998; Peters, 2002; Diffley, 2004). Yet during quiescence, APC^{Cdh1} also plays a negative role in prereplication-complex assembly by targeting the licensing activator Cdc6 for destruction. These opposing roles for APC^{Cdh1} in prereplication-complex formation have generated both interest and controversy (Diffley, 2004). The studies by Mailand and Diffley (2005) (this issue of *Cell*) now provide an explanation for how all three proteins can be APC substrates during quiescence and cell cycle reentry.

The key to the model presented by Mailand and Diffley is the temporal order for reaccumulation of these substrates following quiescence. During quiescence, Cdc6, geminin, and cyclin A are unstable. As cells reenter the cell cycle, Cdc6 begins accumulating before geminin and cyclin A. As a consequence, there is a period of time when Cdc6 is active and induces prereplication-complex formation before the accumulation of the inhibitors that stop the process. But if Cdc6, geminin, and cyclin A are APC substrates and all are recognized equally by the APC ubiquitin ligase, why would production of geminin and cyclin A increase later than

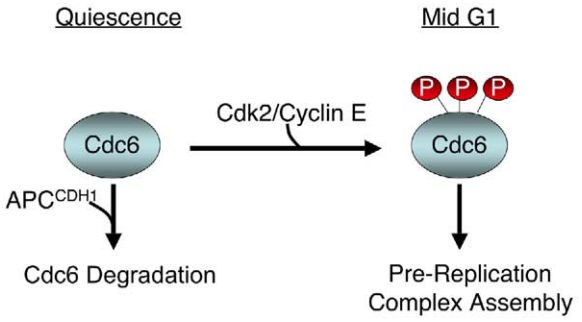

Figure 1. APC^{Cdh1} and Cyclin E-Cdk2 Have Opposing Effects on Cdc6 Stabilization and Prereplication-Complex Assembly during Cell Cycle Reentry from Quiescence

The essential licensing component Cdc6 is degraded during cellular quiescence through the action of APC^{Cdh1}, resulting in inhibition of the assembly of the prereplication complex. Once cells are stimulated to reenter the cell cycle, there is an increase in the activity of the cyclin-dependent kinase cyclin E-cdk2. Cyclin E-cdk2 subsequently phosphorylates the amino-terminal domain of Cdc6 during mid-G1 phase of the cell cycle. Phosphorylated Cdc6 is stabilized because APC^{Cdh1} does not recognize it. Accumulation of Cdc6 induces prereplication-complex assembly before the accumulation of the inhibitors geminin and cyclin A in late G1 phase.

production of Cdc6 during G1? Mailand and Diffley argue that, in fact, geminin, cyclin A, and Cdc6 are not equally recognized by APC^{Cdh1} during cell cycle reentry.

The authors provide evidence that cyclin-dependent kinases (CDKs) such as cyclin E-cdk2 prevent the destruction of Cdc6 by the APC. Most CDKs prevent prereplication-complex formation by inhibiting either the assembly or activity of its components during the S, G2, or mitosis phases of the cell cycle. Meanwhile, other CDKs, such as those containing type E cyclin subunits, do the opposite and promote cell cycle reentry (Geng et al., 2003). In the new study carried out in cultured human cells, inhibition of cyclin E-cdk2 either by treatment with the kinase inhibitor roscovotine or by siRNA depletion of Cdk2 or cyclin E destabilizes Cdc6, suggesting that cyclin E-cdk2 kinase activity is negatively affecting Cdc6 turnover. In this model, stimulating cell cycle reentry causes an increase in cyclin E, which thereby increases the activity of cyclin E-cdk2, ultimately leading to the stabilization of Cdc6 (Mailand and Diffley, 2005; see Figure 1).

This study shows that the mechanism for Cdc6 stabilization involves phosphorylation of Cdc6 by CDKs. Cdc6 contains three serine phosphorylation sites in its amino-terminal domain (Petersen et al., 2000). When the authors mutate these sites to alanine (Cdc6 AAA), Cdc6 is efficiently degraded in the presence of exogenous cyclin E. However, mutation of all three serine residues to aspartic acid (Cdc6 DDD) in order to mimic phosphorylation stabilizes Cdc6 in the presence of excess cyclin E and during quiescence.

These findings prompted the authors to test whether APC^{Cdh1} recognition of Cdc6 is diminished by phosphorylation. This is an especially attractive hypothesis given that the amino-terminal serines of Cdc6 are intermingled with its two APC^{Cdh1} recognition elements, a destruction box and a KEN sequence. Both Cdc20 and Cdh1 have been recently shown to capture the destruction box motif found in APC substrates, potentially bringing substrates into close proximity with the ligase and the E2 (Burton et al., 2005; Kraft et al., 2005). The authors find that, whereas wild-type Cdc6 and Cdc6 AAA interact with Cdh1 as judged by a coimmunoprecipitation assay, the Cdc6 DDD mutant does not associate efficiently. Furthermore, wild-type Cdc6 but not the Cdc6 DDD mutant is robustly degraded in quiescent cell extracts previously characterized as having high APC^{Cdh1} activity (Brandeis and Hunt, 1996; Rape and Kirschner, 2004).

These findings are striking because APC recognition of substrates was thought to be largely independent of substrate phosphorylation status. In fact, this contrasts with work on SCF1, the other major class of ubiquitin ligase that is active throughout the cell cycle. Recognition by the F box component of the SCF ligase targets phosphorylated substrates for degradation (Reed, 2003; Petroski and Deshaies, 2005). Although Cdh1 recognition of Cdc6 is inhibited by Cdc6 phosphorylation, one has to wonder whether other APC substrates are in fact targeted more efficiently for degradation when they are phosphorylated. Furthermore, recent studies have demonstrated that the APC downregulates its own activity based on the availability of substrates during G1 (Rape and Kirschner, 2004). If phosphorylation by a kinase such as cyclin E-cdk2 affects substrate levels, this could potentially affect the kinetics of APC downregulation and would couple two pathways previously thought to be independent.

Future studies will illuminate whether cyclin E-cdk2 affects APC downregulation and whether the APC^{Cdh1} and cyclin E-cdk2 pathways have opposing roles in initiating cell cycle reentry following quiescence. Consistent with these ideas, the loss of the APC subunit APC2 in quiescent hepatocytes induces cell cycle reentry, and cyclin E-deficient cells are unable to reenter the cell cycle from quiescence (Wirth et al., 2004; Geng et al., 2003). If Cdc6 is indeed a major point of convergence for these pathways, one would predict that overexpression of the Cdc6 DDD mutant in cyclin E-deficient cells may stimulate cell cycle reentry, as this mutant stimulates MCM2 loading onto chromatin. We anxiously await the results of these and other experiments to determine whether the APC and cyclin E-cdk2 pathways are indeed globally linked.

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Selected Reading

Blow, J.J., and Dutta, A. (2005). Nat. Rev. Mol. Cell Biol. *6*, 476–486.

Brandeis, M., and Hunt, T. (1996). EMBO J. *15*, 5280–5289.

Burton, J.L., Tsakraklides, V., and Solomon, M.J. (2005). Mol. Cell *18*, 533–542.

Diffley, J.F. (2004). Curr. Biol. *14*, R778–R786.

Geng, Y., Yu, Q., Sicinska, E., Das, M., Schneider, J.E., Bhattacharya, S., Rideout, W.M., Bronson, R.T., Gardner, H., and Sicinski, P. (2003). Cell *114*, 431–443.

Harper, J.W., Burton, J.L., and Solomon, M.J. (2002). Genes Dev. *16*, 2179–2206.

Kraft, C., Vodermaier, H.C., Maurer-Stroh, S., Eisenhaber, F., and Peters, J.M. (2005). Mol. Cell *18*, 543–553.

Mailand, N., and Diffley, J.F.X. (2005). Cell *122*, this issue, 915–926.

McGarry, T.J., and Kirschner, M.W. (1998). Cell *93*, 1043–1053.

Peters, J.M. (2002). Mol. Cell *9*, 931–943.

Petersen, B.O., Wagener, C., Marinoni, F., Kramer, E.R., Melixetian, M., Lazzerini Denchi, E., Gieffers, C., Matteucci, C., Peters, J.M., and Helin, K. (2000). Genes Dev. *14*, 2330–2343.

Petroski, M.D., and Deshaies, R.J. (2005). Nat. Rev. Mol. Cell Biol. *6*, 9–20.

Rape, M., and Kirschner, M.W. (2004). Nature *432*, 588–595.

Reed, S.I. (2003). Nat. Rev. Mol. Cell Biol. *4*, 855–864.

Wirth, K.G., Ricci, R., Gimenez-Abian, J.F., Taghybeeglu, S., Kudo, N.R., Jochum, W., Vasseur-Cognet, M., and Nasmyth, K. (2004). Genes Dev. *18*, 88–98.

Note Added in Proof

A recent paper by Duursma and Agami (2005) (Mol. Cell Biol. *25*, 6937–6947) also reports a link between Cdc6 degradation and cdk2 activity.

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