The CDK-activating kinase Cdk7
Taking yes for an answer

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The cyclin-dependent kinases (CDKs) that control cell division require activation (T) loop phosphorylation for full activity. In metazoans, the only known CDK-activating kinase (CAK) is the Cdk7 complex, which was discovered ~20 y ago and subsequently implicated in CDK activation in vivo in flies and worms. Cdk7 has another essential function as part of transcription factor IIH, to phosphorylate RNA polymerase (RNAP) II and other transcription factors. These two seemingly disparate roles, and the existence of divergent, non-cyclin-dependent CAKs in yeast, initially raised doubts about whether Cdk7 is a CAK in vivo, but no other metazoan enzyme capable of activating CDKs has been identified.[1]

Conclusive evidence that human Cdk7 is a bona fide CAK emerged from chemical genetics—expanding the ATP binding pocket to accommodate bulky adenine analogs that inhibit the resulting analog-sensitive (AS) Cdk7, but not any wild-type kinase. Selective inhibition of Cdk7, in HCT116 colon cancer cells in which wild-type Cdk7 was replaced with Cdk7[as], blocked activation of Cdk1 and Cdk2 and caused arrest at both G_1/S and G_2/M transitions.[2] Cdk7 supports distinct activation pathways for the 2 CDKs, despite their structural similarities; in the case of Cdk1, cyclin binding and T-loop phosphorylation must occur in concert, whereas Cdk2 can be phosphorylated as a monomer.[3] This difference helps ensure activation of Cdk2 before Cdk1, and might obviate the need for a separate CAK that prefers monomeric substrates—a feature of CDK networks in yeast. Moreover, different activation mechanisms might explain why previous studies detected

effects of Cdk7 impairment on Cdk1 but not Cdk2.[1]

Still to be identified was a CAK for Cdk4 and Cdk6, which control cell cycle commitment by phosphorylating the retinoblastoma tumor suppressor protein Rb at the restriction point. It had been suggested that Cdk4 was activated by another CAK, based on instances in which Cdk4 T-loop phosphorylation fluctuates while Cdk7 activity appears constant, and on structural differences between the Cdk4 T loop and those of other CDKs.[4] We showed, however, that Cdk7 is responsible for activation of Cdk4 and Cdk6 through another distinct pathway.[5] Cdk2 and Cdk1 remain phosphorylated for several hours after Cdk7 inactivation, even though their activation de novo is blocked.[2,3] In contrast, Cdk4 and Cdk6 lose activity rapidly upon Cdk7 inhibition in human cells.[5] Differential susceptibility to T-loop dephosphorylation, due to structural differences between Cdk4 and Cdk2 complexes, might place greater demand on a Cdk4-activating kinase to overcome antagonism by phosphatases and provide an opportunity for regulation during G_1. Consistent with this scenario, activation of Cdk4 (but not Cdk2) in vitro was stimulated by phosphorylation of Cdk7's own T loop. In vivo, Cdk7 T-loop phosphorylation increased during G_1 when quiescent cells were stimulated by mitogens—the first evidence that changes in CAK activity might regulate a key cell cycle transition.

Nonetheless, doubts (and doubters) persist. Another study in Cdk7[as] HCT116 cells showed that Cdk7 inhibition caused rapid inactivation of Cdk4 and Cdk6 (in agreement with our results) but loss

of Cdk4 T-loop phosphorylation only in the population bound to the CDK inhibitor p21.[6] This was taken as evidence that another CAK works on p21-free Cdk4, even though it cannot support Cdk4 activity or cell cycle progression in Cdk7[as/as] cells treated with allele-specific inhibitors. Moreover, there was no demonstration that p21-free Cdk4 became phosphorylated when Cdk7 was inactive; it might simply remain so, possibly due to different rates of dephosphorylation in distinct kinase sub-populations, for which Cdk2 provides a precedent.[3]

In another study, conditional disruption of Cdk7 in mouse embryonic fibroblasts (MEFs) blocked cell division and activation of Cdk1, Cdk2, Cdk4, and Cdk6. Proliferation and CDK T-loop phosphorylation were maintained in Cdk7[mut/mut] MEFs, however, when an SV40 large T antigen fragment was expressed to inactivate the pocket proteins Rb, p107 and p130.[7] This seems to challenge the "single-CAK theory" and provide evidence for a cryptic, Cdk7-independent CDK activation pathway normally suppressed by Rb. This pathway remains hypothetical, however, until the responsible activity is detected and proven not to be due to the residual Cdk7 complexes in extracts of Cdk7[mut/mut] MEFs rescued by T antigen (or by "CAK-bypass" variants of Cdk1 or Cdk2).[7]

Is Cdk7 the major CAK in metazoans? The answer, from recent chemical-genetic and knockout studies, as well as older "classical" genetics, is an unequivocal "yes", which is corroborated by demonstrations that removal or chemical inhibition of Cdk7 abolishes CAK activity of whole-cell extracts.[1,8] Cdk7 is a common

activator of cell cycle CDKs, but its CAK function is not restricted to the cell cycle machinery: one of its targets, Cdk9,[8] is part of positive transcription elongation factor b, which regulates elongation and maturation of RNAP II transcripts.

Whether Cdk7 is the only CAK is likely to remain an open question. In the 2 decades since its discovery, however, there have been several, ultimately unsubstantiated challenges to Cdk7's position as the major CAK in vivo. Despite differences in interpretation, the 3 recent studies discussed here do not differ on a key point: inactivating Cdk7, either by chemical inhibition[5,6] or gene disruption,[7] causes general failure of activating phosphorylation affecting G_1, S phase, and mitotic CDKs. For now then, Cdk7 is the only CAK we know, and novel insights continue to emerge from studies that focus on how it activates different CDKs at the right time and place.

References

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