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Cdk7
Open questions beyond the prevailing model

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Attainment of full activation of protein kinases, including cyclin-dependent kinases (Cdks), entails the phosphorylation of a key residue. Cdks are heterodimeric complexes consisting on a regulatory subunit (cyclin) that activates and provides substrate specificity to the catalytic counterpart. However, cyclin binding perse is not sufficient to grant maximal activity requiring an additional step. Cyclin binding provokes the repositioning of the activation segment (T-loop), inducing the exposure of a particular threonine that upon phosphorylation allows full activation. Therefore, T-loop phosphorylation by a Cdk-activating kinase (CAK) is mandatory for Cdk function. Paradoxically, in metazoans this CAK is another Cdk (Cdk7) that together with cyclin H and Mat1 form the Cdk activating complex.[1] Yet during the previous decade, various reports described the identification of Cdk7-independent CAK activities.[2] This, together with the lack of a consistent mammalian system, questioned the notion that the cellular CAK is entirely provided by Cdk7. The generation of HCT116 cells carrying ATP analog-sensitive *Cdk7* alleles (Cdk7^{as}) provided a genetic tractable system and was used in a series of elegant papers by the Fisher laboratory to exclude the existence of additional CAKs.[3] It also uncovered that the activity and substrate preferences of Cdk7 govern the pairing rules for Cdk/Cyclin complexes. Cdk7 recognizes Cdk1/2 with different kinetics, resulting in a built-in mechanism controlling the hierarchy of Cdk-Cyclin binding in terms of specificity and timing.[4]

The essential CAK function of Cdk7 has recently been confirmed in vivo by classical genetics.[5] Cdk7 elimination in mouse tissues results in T-loop hypophosphorylation and proliferation arrest. Yet, mouse embryonic fibroblasts devoid of Cdk7 resume cell proliferation with wild type kinetics upon inactivation of the Retinoblastoma (RB) family. Interestingly, T-loop phosphorylation of Cdk1/2 is also rescued, illustrating the existence of alternative CAK pathways independent of Cdk7 (5). It is, however, unclear how this is mechanistically brought about. Cdk2 (but not Cdk1) autophosphorylates in vitro.[6] Whether this also occurs in vivo and whether Cdk1/2 T-loop phosphorylation might be triggered by deregulated E2F activity is unknown. Anyhow, these CAK-alternative pathways are not operative during normal homeostasis, since highly proliferative tissues cease to proliferate and display T-loop hypophosphorylation upon elimination of Cdk7 (5).

The CAK complex is also a component of the transcription factor TFIIH and mediates the phosphorylation of serine residue 5 (S5) within the C-terminal domain (CTD) of RNA polymerase II (RNA pol II), thereby regulating promoter clearance and progression from the pre-initiation stage. Unlike its Cdk-activating function, several reports suggested that Cdk7 is dispensable to provide the TFIIH-associated kinase, probably due to compensatory functions exerted by related kinases, a situation evocative of the substantial redundancy reported for the Cdks involved in cell cycle control. In the Cdk7$^{as/as}$ cells, Cdk9 (and possibly Cdk8) cooperate in the phosphorylation of CTD S5 in a gene-dependent manner. Cdk7 inhibition did not induce a major transcriptional disruption but altered chromatin modifications associated with transcription elongation (such as H3K36 trimethylation or H4 acetylation) and caused abnormal RNA pol II pausing at the promoter-proximal and poly(A) sites.[7] Since these effects were analyzed short-term in cultured cells, the possibility that sustained Cdk7 inhibition might substantially perturb transcription could not be discarded. Yet, adult mouse tissues show normal S5 phosphorylation and expression of tissue-specific markers up to 8 months after genetic ablation of Cdk7 (5). This is reminiscent of the outcome upon Mat1 elimination in Schwann cells.[8] Since the steady-state level of the three CAK components is interdependent ,this suggests that they are concomitantly dispensable for RNA pol II function in postmitotic cells.

Cdk7-mediated modulation of RNA pol II might be required for the precise coordination of specialized transcriptional programs, yet overtly dispensable for housekeeping functions. Indeed, there is precedent for differential recruitment of Cdk7 to promoters. Transcription of heat shock but not of histone genes is affected by a Cdk7 mutation.[9] Furthermore, Cdk7 is specifically recruited to the *p21^{Cip1}* promoter depending on the type of DNA damage. Yet, irrespectively of Cdk7 recruitment, the overall levels of S5 phosphorylation on this promoter were similar.[10] In addition to being regulated by various kinases and phosphatases, the CTD is also methylated, and several lysine residues are potential sites for acetylation, sumoylation and ubiquitylation. Thus, the CTD is a dynamic scaffold providing

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a readable code that may be modified appropriately for the fine-tuning of gene (and context)-specific transcription.[11]

Finally, Cdk7 has been considered a target for cancer therapy, and inhibitors such as SNS-032 are in phase I clinical trials. Nonetheless, the supposed toxicity caused by the transcriptional disruption upon Cdk7 inhibition has been of concern. We have shown that efficient elimination of Cdk7 in vivo is detrimental for the homeostasis of highly proliferative tissues without affecting global transcription in non-dividing cells.[5] Since Cdk7 heterozygote animals are normal, and tumor cells display elevated levels of Cdk7, any clinical benefit would depend on a therapeutic window specifically affecting CAK function in cancer cells. Importantly, a putative CAK-alternative mechanism was found to be in operation upon inactivation of the RB pathway.[5] Since this scenario is applicable to numerous human cancers, it should be taken into consideration, as it may facilitate drug-resistance to therapies based on Cdk7 inhibitors.

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