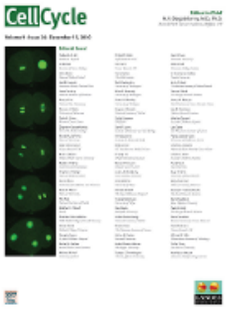

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CXCR4 and renal cancer: From lab to bedside

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Cell Cycle News & Views

Cyclin E goes nuts: A cell cycle regulator affects male fertility

Comment on: Liberal V, et al. Cell Cycle 2010; 9:4222–7.

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In the 1980s and early 1990s, at the hay-day of the discovery of cyclins and cyclin-dependent kinases (CDKs) in yeasts and mammalian organisms, a picture of the cell cycle emerged in which progression through the different stages was "pushed" by sets of specialized CDKs: D-cyclins and CDK4/CDK6 (G1), E- and A-cyclins and CDK2 (S and G2) and B-cyclins and CDK1 (mitosis). As drivers of cell proliferation, most cyclins and CDKs were anticipated to display oncogenic potential. Indeed, misregulation of cyclins, CDKs and CDK inhibitors is a common occurrence in human cancers.[1] Overexpression of E-cyclin mRNA and protein is particularly frequent and has prognostic significance in some cases.[2]

In recent years, the classic view of the cell cycle has been challenged by the surprising discovery that mouse strains lacking individual cyclins or CDKs are viable. Strikingly, even a CDK2/CDK4/CDK6 triple knock-out embryo can undergo millions of mitotic divisions and develop up to 12.5 days of gestation.[3] These genetic studies indicate that only cyclin B-CDK1 is strictly required to drive the mitotic cell cycle, while the rest of CDKs may still play a physiological role but are only essential in specialized cell types.[1] Consistent with this new scenario, both isoforms of cyclin E, E1 and E2, are individually dispensable in the mice.[4,5] However, transgenic mice overexpressing cyclin E1 can develop tissue hyperplasia and carcinomas in the mammary gland.[2] In human cell lines, deregulation of cyclin E1 interferes with DNA replication[6] and promotes genomic instability.[7] Therefore, the issue of whether cyclins and CDKs are oncogenic and could make good targets for anti-cancer therapy is still a relevant one.[1]

In a previous issue of *Cell Cycle*, the group of Steve Reed at Scripps Research Institute (La Jolla, CA), one of the discoverers of human cyclin E, reports the generation of a new transgenic mouse model in which a proteolysis-resistant version of cyclin E1 is ectopically expressed in testicular germ cells.[8] Transgenic mice are born at the expected ratios, have a normal lifespan and do not develop detectable neoplastic lesions in the testis. A first implication of this result is that overexpression of cyclin E has limited oncogenicity in vivo, at least in this organ. This observation is actually in line with previous data from other cyclin E transgenic models. The mammary carcinomas observed after ectopic cyclin E expression occurred only in a small fraction of the animals and after a long latency period. Besides, deregulated expression of cyclin E in T cells led to lymphomas only when combined with mutagenic chemicals or with loss of p27 (reviewed in ref. 2). The likely explanation for these effects is that the pro-transformation potential of cyclin E is only unleashed in cooperation with other oncogenic events.

But even if misregulation of cyclin E alone is not necessarily oncogenic, it is far from harmless. In this new mouse model, the unexpected consequence was male infertility due to partial testicular atrophy, incomplete development of the seminiferous tubules and defective spermatogenesis. How a situation of cyclin E "gain of function" could lead to these effects is still not fully understood, but it could entail a combination of mitotic and meiotic defects. On one hand, the authors find a defect in spermatogonial mitotic proliferation in testes shortly after birth, which could promote the formation of aberrant "Sertoli cells-only" tubules in the adult transgenic mice.[8] On the other hand, meiotic cell cycles depend heavily on E-cyclins and CDK2, their canonical partner. Ablation of cyclin E2 leads to testicular atrophy and reduced male fertility6 and loss of CDK2 makes both male and female mice sterile.[9] In CDK2$^{-/-}$ males, spermatocytes show incomplete chromosomal pairing and are arrested at the pachytene stage due to the accumulation of double-strand breaks.[10] With these antecedents, it is conceivable that meiotic cell cycles are also sensitive to cyclin E overexpression. This new transgenic mouse strain8 provides a valuable tool to study the impact of cyclin/CDK misregulation on the mitotic and meiotic germ cell cycles and its ultimate consequences for fertility.

Acknowledgements

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When enough is enough: Detrimental effects of excess histones

Comment on: Singh RK, et al. Cell Cycle 2010; 9:4236–44.

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The primary function of histones is to help package DNA through formation of nucleosomes and higher-order chromatin structures. Proliferating cells are confronted with the daunting task of coordinating histone and DNA synthesis. During S phase, cells strive to deposit newly synthesized histones immediately behind DNA replication forks.[1] Several lines of evidence suggest that rapid packaging of nascent DNA into nucleosomes is important to minimize chromosome rearrangements and promote cell survival in response to spontaneous and genotoxic agent-induced DNA lesions that impede replication.[2,3] However, rapid synthesis of new histones to achieve timely packaging of nascent DNA into nucleosomes is an inherently risky business. This is because the total rate of DNA synthesis declines from early to late S phase and abruptly drops when DNA lesions impede replication and activate the intra-S DNA damage checkpoint that suddenly inhibits firing of new replication origins.[4] These two physiological conditions lead to an accumulation of excess histones that are not packaged into chromatin.[5] Because the four canonical core histones (H2A, H2B, H3 and H4) are highly basic proteins (isoelectric points between 11 and 12), they have the potential to bind non-specifically to negatively charged macromolecules that carry genetic information, namely RNA and DNA.

Previous studies had revealed that excess histones elevate the incidence of spontaneous mitotic chromosome loss and are cytotoxic to cells treated with genotoxic agents.[5,6] However, the molecular mechanisms by which excess histones exert their deleterious effects had not been extensively investigated. In a previous issue of *Cell Cycle*, Gunjan and colleagues demonstrate that excess histones bind non-specifically to RNAs, perturb chromatin structure and alter the expression of roughly 4% of yeast RNAs.[7]

Fortunately, cells have evolved an arsenal of mechanisms to attenuate the adverse consequences of excess histones. These include histone chaperones involved in nucleosome assembly, which provide a first line of defence by binding to new histones, thus preventing them from interacting non-specifically with nucleic acids. In addition, at least three other cellular responses act in a concerted fashion to limit the accumulation of excess histones in response to DNA damage during S phase: histone gene repression, degradation of histone mRNAs, and degradation of excess histone proteins.[8,9] The latter has thus far only been documented in *S. cerevisiae* where degradation of excess histones depends upon the DNA damage response kinase Rad53 (a kinase functionally related to human CHK1 and CHK2).[5] Whether a related mechanism also exists in human cells is currently unknown. Hopefully, the findings reported by Gunjan and colleagues will stimulate further studies of histone homeostasis pathways that contribute to the DNA damage response and the maintenance of genomic stability.

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Skirmish between the mighty p53 protein and the complex retrovirus HIV-1

Comment on: Mukerjee R, et al. Cell Cycle 2010; 9:4569–78.

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Human immunodeficiency virus type 1 (HIV-1), a causative agent of AIDS, belongs to a group of viruses known as complex retroviruses. The genetic features that distinguish this group of retroviruses from others (otherwise known as simple retroviruses) are the presence of additional genes besides the structural gag, pol and env in the viral genome. Professor Dorothy Crawford, in describing retroviruses, has aptly noted that retrovirus is forever.[1] The replication mode of retroviruses involving an integrated form of viral DNA (proviral DNA) in the host genome ensures that the virus is there as long as the cell is alive. Studies on viruses including retroviruses have provided valuable information about the regulatory mechanisms in prokaryotic and eukaryotic cells. The paper published in a previous issue by Mukerjee et al. (2010) sheds light on control and counter control by viral and cellular genes tipping the balance in favor of the virus to thrive.[2]

HIV-1 is self-reliant to an extent. The virus carries multiple enzymes in the particle for the events associated with virus infection of appropriate target cells and replication. The viral enzymes are reverse transcriptase, integrase and protease, which mediate reverse transcription of viral RNA to DNA, integration of viral DNA and maturation of virus, respectively. However, the virus also needs additional help from the host cells for completing the replication cycle. The cellular factors in the infected cells interact with the viral proteins and/or the signal sequences in the viral genome to aid the virus. Such interactions, in the context of HIV-1, result in either productive viral replication or a latent state of genome in the infected cells.

To successfully infect and survive in the host, the virus has to have multiple strategies. The immune system of the host is known to control the infectious agents entering the body. HIV-1 genome, like several other viruses, codes for proteins that interfere with the immune response of the host and also carries such proteins in the virus particle. In addition to overcoming this hurdle, the virus also needs help from the host cells in terms of transcription and translation for its replication. In this regard, HIV-1 has to deal with the control exerted by cellular proteins including p53. Given its notoriety as a pleiotropic protein, p53 is involved in cell cycle regulation, differentiation and apoptosis. Earlier studies have shown that p53 protein activates the transcription of genes and also represses gene expression in some cases.[3] HIV-1 promoter located in the long terminal repeat (LTR) region of viral DNA falls in the latter category.[4]

Mukerjee et al. (2010) utilized the state-of-the-art technologies to study the interactions between p53 and HIV-1. The authors undertook the studies based on an earlier observation from the laboratory that cdk9 induced p53.[5] These results have led them to the questions outlined in the present article. Considering the extent of neurological disorders in HIV-1 infected individuals, the authors have used CNS derived primary astrocytes to assess the effect of p53 on HIV-1 LTR directed transcription involving a reporter plasmid construct. Interestingly, wild-type p53 reduced transcription. Further, primary microglia infected with HIV-1 JR-FL in combination with the expression of p53 through a viral vector (adenovirus) also exhibited a delay in virus replication. Any time you deal with a protein like p53 which is linked to apoptosis, cell death may also have to be looked at closely in the cell culture system used for analysis. The authors confirmed that the delay in virus replication was not due to cell death by carrying out experiments using U937 cells infected with HIV-1 and PARP as a read out protein. The reduced viral transcription and delayed viral replication may be related to the prevention of phosphorylation of CTD of RNA polymerase II. Studies conducted in the authors' laboratories showed supporting evidence from the point of view of initiation, elongation and chromatin organization. The experiments further show that the cellular factor Pirh2 interacts with p53 resulting in the attenuation of p53 effect.

The authors conclude enthusiastically that p53 may be a potential target gene for controlling HIV-1. In the absence of a vaccine against HIV-1 and the continued emergence of resistant viruses in the face of antiviral therapeutic agents, reports about novel cellular and viral targets are welcome news. However, such a goal clearly needs additional work.

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CXCR4 and renal cancer: From lab to bedside

Comment on: D'Alterio C, et al. Cell Cycle 2010; 9:4492–4500.

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Recently, it was shown that loss of VHL function results in strongly enhanced transcription of HIF-α-inducible genes, especially in up-regulation of CXCR4.[1] Therefore, the VHL tumor suppressor gene product is one of the major regulators of CXCR4 expression and increased CXCR4 expression levels are most likely a consequence of impaired VHL function in clear cell RCC. Strong CXCR4 expression is associated with poor prognosis of RCC.[1]

Thus, by expressing CXCR4, tumours acquire properties that enable them to invade tissue barriers, migrate to secondary organs, and form metastases. Although CXCR4/CXCL12 expression patterns may explain selection of specific organs for the formation of metastasis, the exact molecular mechanisms by which the CXCR4/CXCL12 axis promotes tumour invasion are still unclear. Other pVHL functions include fibronectin matrix assembly, p53 stabilisation, and transactivation. In addition, pVHL has the ability to bind and stabilise microtubules by protecting them from depolymerisation, which is a prerequisite for cilium formatio.[2] In fact, two previous in vitro studies showed that by re-expressing pVHL in VHL null clear-cell RCC cell lines, pVHL regulates the formation of primary cilia.[2] These observations strongly suggest that loss of VHL function in renal epithelial cells leads to degeneration of primary cilia, which represents a critical step towards cyst formation and clear cell RCC development in patients with VHL. Interestingly, renal cysts are also present in about 60% of individuals suffering from the VHL disease. One might hypothesize that cyst formation is one of the first visible renal alterations in VHL-caused tumor formation. The elucidation of the different pVHL functions is the basis for understanding the novel therapeutic strategies for patients with RCC. It is theoretically possible to target the VHL pathway for therapeutic intervention at different levels. VHL protein function could be replaced, restoring binding to HIF-1 and allowing its proteasomal degradation. Furthermore, the activity of HIF-1 could be a target for inhibition. Finally, molecules up-regulated by HIF-1 (as CXCR4) also provide specific targets for potential downstream inhibition of the VHL pathway.

Enhanced CXCR4 expression is driven by HIF-1α and predicted poor tumour specific survival.[1] More recently, CXCR4 was detected on circulating cytokeratin positive RCC cells from patients with known metastatic disease. Also, the CXCR4 expression on RCC cells was found to directly correlate with the ability to metastasize in vivo in models of human RCC. CXCR4 expression may define increased metastatic potential of RCC, potentially affecting survival. Additionally, CXCR4 was also described as a stem cell marker.[3] In bone marrow the mesenchymal-stromal cells represent the major source of CXCR4 ligand, CXCL12. The CXCR4-CXCL12 axis acts as a migration mechanism and is related to tissue hypoxia and repair of hypoxic damage. The HIF-1 transcription factor induces the local expression of CXCL12, which attracts circulating progenitor cells for tissue repair. In hypoxic tumors or in tumors that display mutations in the von Hippel–Lindau tumor suppressor protein pVHL, HIF-1 upregulates CXCR4 expression providing a survival benefit for tumor cells with high CXCR4 expression.[3]

The paper from D'Alterio et al.[4] shows that an increased expression of CXCR4 in RCC correlates with symptomatic and advanced stages of disease and is independently associated with recurrence-free survival, thereby confirming its potential role in the acquisition of more aggressive tumor features.[1] CXCR4 is therefore a potential interesting prognostic marker for RCC. Its expression can be assessed on tumor specimens after nephrectomy and potentially also on tumor biopsies performed to evaluate tumor histology before treatment decision. In fact, core biopsies of renal tumors performed with appropriate techniques have been shown to be safe and provide sufficient tissue for a proper histologic evaluation, including immunohistochemical staining.[5] CXCR4 may be integrated in the future with other clinical and histological variables in new improved integrated prognostic models for treatment decision making, patient counseling, planning of individualized surveillance protocols and patient selection for clinical trials[6] Finally and very importantly, the results of this study shed new light on the potential use of CXCR4 inhibitors (AMD3100) as a new therapeutic strategy for the treatment of metastatic RCC or of locally advanced/high risk RCC in an adjuvant setting after nephrectomy.

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Research highlights on a notable retrovirus and a popular guardian gene

Comment on: Mukerjee R, et al. Cell Cycle 2010; 9:4569–78.

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HIV is a lentivirus believed to have been occasionally infecting humans since the beginning of the 20th century. After the first recorded appearance of HIV/AIDS in 1981, more than 25 million people have died in a worldwide epidemic and an unprecedented effort has been put in place to contain the virus. HIV continues to spread unabated since many therapeutic strategies have failed. For instance the envelope gp120 or Env-based vaccine failed to protect volunteers in phase III clinical trials.[1]

The p53 transcription factor is also described as "the guardian of the genome" or the "master watchman," referring to its role in conserving stability by preventing genome mutation. p53 regulates cell cycle, apoptosis, senescence, DNA repair and cell metabolism. Activity of p53 is lost in human malignancies either by p53 mutations or by loss of cell signaling upstream or downstream of the gene. MDM2 is an important negative regulator of tumor suppressor p53 and acts by recognizing its N-terminal trans-activation domain (TAD), inhibiting p53 transcriptional activity, and promoting p53's ubiquitination and degradation. Furthermore, Pirh2,[2] COP1, and ARF-BP1/Mule ubiquitin-protein ligases, like Mdm2, participate in an autoregulatory feedback loop that controls p53 function.

Cdk9 or cyclin-dependent kinase 9 is a part of the multiprotein complex TAK/P-TEFb, which is an elongation factor for RNA polymerase II-directed transcription and phosphorylates the C-terminal domain of the largest subunit of RNA polymerase II. This protein forms a complex with and is regulated by its regulatory subunit cyclin T or cyclin K. HIV-1 Tat protein was found to interact with this protein and with cyclin T, suggesting a key role in AIDS pathogenesis.

Recently Mukerjee and coworkers[3] explored the contribution of wild-type and mutant p53 (S392A) in HIV-1 transcription (HIV-1 group M, most common and pathogenic strain of the virus) using as models human glial cells (non-neuronal cells), specifically primary microglia and astrocytes (the most abundant type of macroglial cells). The authors showed that only in the presence of wild-type p53 is transcriptional elongation interrupted in HIV-1, as assessed by chromatin immunoprecipitation (ChIP) and elongation assays.[3] Upregulation of p53 wild type decreases phosphorylation of serine 2 but not serine 5 of the carboxyl terminal domain (CTD) of RNA polymerase II of HIV-1. Notwithstanding the pause of viral transcription and replication, the effects of p53 are not dramatic, possibly due to the action of Pirh2 on p53. Indeed, the authors propose the following mechanistic scenario: cdk9 activates Pirh2 causing its dephosphorylation, and enhanced interaction between Pirh2 and p53, and then ubiquitin-mediated proteolysis of p53. Very interestingly, p53 may inhibit viral replication without causing cell death. Indeed, in cultured U937 human macrophage cells either infected with MΦ-tropic HIV-1 JR-FL strain[4] or cotransfected with HIV-1 JR-FL and adenovirus expressing p53 wild type, p53 does not increase the endogenous cleaved PolyADP-ribose polymerase (PARP) expression levels, a programmed cell death indicator. All these aspects point to potential exploitation of the p53 pathway in the therapeutic management of AIDS. However, limitations would be expected to arise since p53 is a central molecule in cell homeostasis.

p53 is a key molecule involved in multiple aspects of cell cycle control and widely considered as a suitable target in relation to gene and immuno-based treatments in cancer. We are expecting to learn more in the near future on Pirh2 and p53 inhibition since for now this is a hypothesis that needs more experimental support. The data presented by Mukerjee and collaborators[3] suggest that widespread p53 activation in glial cells may be an important target for therapeutic intervention of "neuroAIDS," a term that refers to clinical syndromes such as sensory neuropathy, myelopathy, HIV dementia, and cognitive/motor disorder. Strains of mutant HIV in the U.S. and Europe are expanding[5] and use of small molecules that alter the function of host's proteins, such as p53 or cdk9 may be a viable option.[6] For HIV patients it is vital to open the door to a range of new experimental and therapeutic possibilities. However, these should be supported by sound scientific evidence, especially since effective ways to slow the progression of disease are presently available.[7]

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Linking Cdc7 with the replication checkpoint

Comment on: Matsumoto S, et al. Cell Cycle 2010; 9:4627–37.

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Replication fork stalling leads to activation of conserved signal transduction pathways that integrate DNA synthesis with cell cycle progression and help maintain genome stability. In *S. pombe* nucleotide pool depletion using hydroxyurea (HU) elicits activation of the proximal checkpoint protein kinase Rad3 (equivalent to human ATR) which phosphorylates a mediator protein Mrc1 (Claspin analog in humans) leading to activation of the effector checkpoint kinase Cds1 (homologous to human Chk2 but functionally equivalent to human Chk1).[1] Activated Cds1 slows DNA replication, stabilizes stalled replication forks and prevents premature entry into mitosis.

Hsk1 (equivalent to Cdc7 in humans) is an essential protein kinase that is required for the initiation of DNA synthesis. Mcm2, Mcm4 (components of the Mcm2-7 helicase complex) and perhaps other replication factors are important Cdc7 substrates which, when phosphorylated, promote recruitment of the initiation factor Cdc45 to licensed origins of replication.[2] Thus Cdc7-dependent Cdc45 recruitment is crucial for establishing replication forks during S phase. Studies in several experimental systems have implicated roles for Cdc7 in mediating checkpoint activation and conferring tolerance of replication stress.[3,4] However, the precise relationship between Cdc7 and other components of replication stress-induced pathways is not well understood.

An exciting new study by Masai and colleagues suggests novel roles for Hsk1 in regulating Mrc1 phosphorylation in response to replication stress (Matsumoto et al., 2010). These authors report that the temperature-sensitive *hsk1-89* mutation reduces HU-induced phosphorylation of Mrc1 and Cds1, thereby establishing that Hsk1 contributes to the checkpoint signaling response. To test whether Cdc7 affects maintenance of Mrc1 phosphorylation, the authors activated the checkpoint at the permissive temperature (when Hsk1 is active), then compared the magnitude and duration of HU-induced Mrc1 phosphorylation at both restrictive and permissive temperatures. Interestingly, at the non-permissive temperature (when Hsk1 activity was compromised), Mrc1 phosphorylation was not sustained. Therefore Hsk1-dependent maintenance of Mrc1 phosphorylation is dissociable from its role in establishing replication forks.

IP-kinase assays showed that Rad3 immunoprecipitated from hsk1-89 cells retains full activity towards Mrc1 in vitro (indicating that reduced Mrc1 phosphorylation in the absence of Cdc7 is not due to reduced Rad3 kinase activity). Consistent with the possibility that Hsk1 might contribute to HU-induced Mrc1 phosphorylation directly, in vitro kinase assays demonstrated that Hsk1 phosphorylates Mrc1 at sites distinct from SQ/TQ clusters targeted by Rad3. Further work is necessary to fully test the significance of direct Hsk1-mediated Mrc1 phosphorylation in vivo.

To explore possible mechanisms of Cdc7-dependent Mrc1 phosphorylation the authors examined checkpoint activation in temperature-sensitive strains harboring mutations in other replication factors. Interestingly, HU-induced Mrc1 phosphorylation and Cds1 activation were abrogated in *goa1* (Cdc45), but not in *cdc19* (Mcm2) or *cdc20* (Polϵ) mutants. Therefore, Cdc45 mutations specifically compromise activation of the Rad3-Mrc1-Cds1 checkpoint pathway. Imperfect fork structures generated when Hsk1 activity is limiting or in the presence of a mutated Cdc45 may be unable to support checkpoint activation. The continuous requirement of Hsk1 for Cds1 activation could indicate that in cells experiencing replication stress Hsk1-dependent phosphorylation event(s) are required to maintain Cdc45 at the replication fork.

It should be noted that Mrc1-null yeast cells are sickly but their growth rate can be restored by inactivation of histone deacetylases.[5] This result suggests that tightly packed heterochromatin induces replication stress that requires Mrc1 for resolution. Thus, the Rad3-Cdc7-Mrc1 signaling pathway may be induced regularly during S phase when heterochromatic DNA is encountered by DNA replication forks.

Precisely how Cdc45 mutations compromise Mrc1 phosphorylation is an important question posed by this study. Mrc1 is a component of the Replication Fork Protection Complex (RFPC, comprising Mrc1, Swi1, Swi3 and Mcl1 in *S. pombe*) which couples replicative helicase and DNA polymerase activities.[6] The RFPC likely provides a platform for activation of checkpoint kinases and for initiation of DNA damage signaling.[7,8] Potentially, interactions between the Cdc45-containing replicative helicase complex (Cdc45-Mcm2-7-GINS) and the RFPC might be required to support Rad3-mediated Mrc1 phosphorylation and Cds1 activation. Defective Cdc45 encoded by the mutant *goa1* allele may not support helicase-RFPC interactions that are necessary for Mrc phosphorylation by Rad3.

Intriguingly, Cdc7 and Cdc45 have previously been implicated as negatively-regulated distal targets of S-phase checkpoint signaling,[9] yet as shown by the new study both proteins also appear to play key proximal roles in facilitating and maintaining checkpoint signaling. This work will certainly prompt further experiments to probe the mechanisms by which Cdc7 and Cdc45 contribute to the different elements of S-phase checkpoint signaling.

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