Uninhibited CDK2 Exposes Differences in Centrosome and DNA Copy Regulation

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Cyclin-dependent kinases (CDK) are responsible for activating cellular machinery that drives cell cycle progression. As their name implies, they are managed by cyclin proteins that guide CDKs to their appropriate targets and enhance their activity. CDK1, which orchestrates the G2 and M cell cycle phases with the assistance of cyclins A and B, also serves as a molecular 'stop/go' switch between G2 and M phases. When CDK1 residues 14 (threonine) and 15 (tyrosine) are phosphorylated, CDK1 activity is inhibited. When these modifications are removed by members of the CDC25 phosphatase gene family, CDK1 is activated. The signals that control this phosphorylation 'switch' safeguard the order and timing of cell cycle events. For example, checkpoint pathways that sense unreplicated DNA promote inhibitory CDK1 phosphorylation to delay or prevent cell division. Previous studies have shown that if these phosphorylation sites are removed by mutating residues T14 and Y15 to alanine and phenylalanine, cells expressing CDK1AF are rendered insensitive to checkpoint pathways.

Although less is known about CDK2, which enables G1 and S phase progression and associates with cyclins E and A, the same cellular components that phosphorylate/dephosphorylate CDK1 inhibit/activate CDK2 at residues T14 and Y15. This led postdoctoral fellow Dr. Hui Zhao and former colleagues in Dr. James Robert's lab of the Basic Sciences Division to inquire whether CDK2 inhibitory phosphorylation serves as a similar checkpoint-sensitive 'stop/go' switch between G1 and S phases. To address this question, they analyzed several phenotypes relating to the cell cycle progression in mouse embryonic fibroblasts (MEFs) homozygous for a knock-in variant of CDK2 whose inhibitory phosphorylation sites have been removed by mutating residues T14 and Y15 to A14 and F15 (CDK2AF).

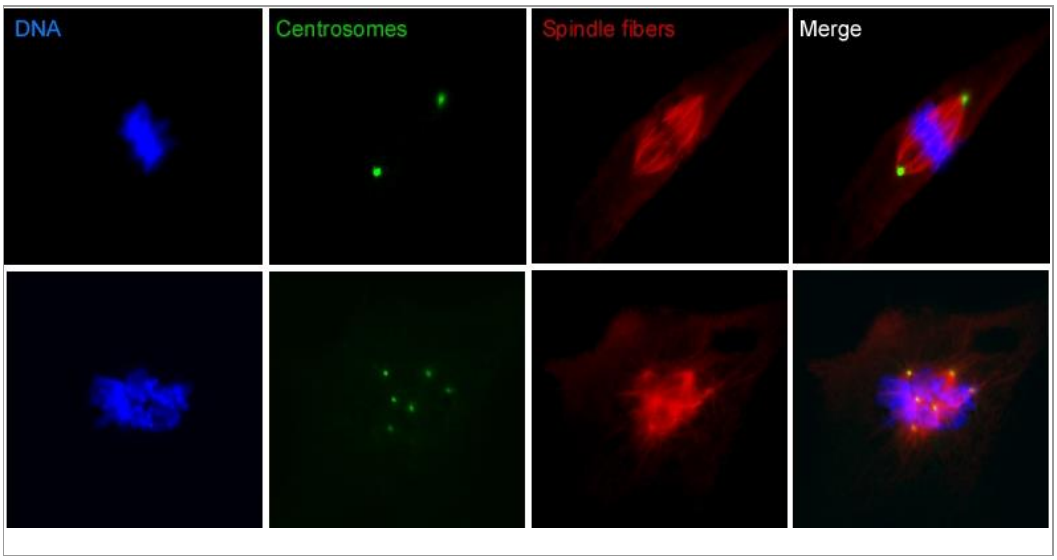
Unexpectedly, CDK2AF MEFs delayed S-phase entry to a similar extent as wild-type MEFs following UV-exposure, suggesting that CDK2AF cells remain sensitive to DNA damage. This was the first clue that the G1 to S phase transition involves CDK2 in a way that differs from the G2 to M phase regulation by inhibitory phosphorylation of CDK1. Zhao *et al.* then postulated that the CDK2 inhibitor p21, which was upregulated through DNA damage sensing pathways, might have bound to CDK2AF and prevented it from bypassing checkpoint pathways in irradiated cells. However, when p21

expression was knocked-down in CDK2AF MEFs, the resulting cells again delayed S-phase entry upon UV exposure to a similar extent as wild-type MEFs. As cyclin E-associated CDK activity was intact (even elevated) in these cells, Zhao *et al.* have focused their ongoing studies on the regulation of cyclin A-associated CDK2 activity, as cyclin A-CDK2 can initiate S phase in the absence of cyclin E.

Together these results suggest that CDK2 inhibitory phosphorylation does not unilaterally control DNA replication. In contrast, CDK2 inhibitory phosphorylation does appear to serve a non-redundant role in controlling centrosome duplication, as 10-15% of synchronized CDK2AF cells exhibit four or more centrosomes, compared to only 5% of synchronized controls.

Although it remains to be shown if there is a singular signaling pathway that underlies the decision to enter S phase and if so, what the nature of that 'stop/go' switch is, this study has demonstrated that DNA replication employs many fail-safes to prevent genetic instability caused by DNA damage.

Zhao H, Chen X, Gurian-West M, Roberts JM. 2012. Loss of cyclin-dpendent kinase 2 (CDK2) inhibitory phosphorylation in a CDK2AF knock-in mouse causes misregulation of DNA replication and centrosome duplication. *Molecular and Cellular Biology*, Epub ahead of print, doi: 10.1128/ MCB.06721-11



Images courtesy of Hui Zhao

Mouse embryonic fibroblasts (MEFs) that express a deregulated variant of cyclin-dependent kinase 2 (CDK2) exhibit an increased number of centrosomes per cell (bottom row) compared to wild-type MEFs (top row).