ANTIOXIDANTS & REDOX SIGNALING
Volume 7, Numbers 5 & 6, 2005

Forum Editorial

Redox Control of the Cell Cycle: A Radical Encounter

EMILY C. ROTHSTEIN[1] and PAMELA A. LUCCHESI[2]

OXIDATIVE STRESS elicits diverse cellular responses ranging from cell proliferation and transformation to apoptosis and senescence. These vastly different effects are likely dependent on the types and amounts of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that are generated, the duration of the oxidative burst, the cellular antioxidant defense systems, and the cellular context in which oxidative stress occurs (9). For example, exposure of cells to low amounts of hydrogen peroxide (H_2O_2) have been shown to mediate platelet-derived growth factor (PDGF)-induced vascular smooth muscle proliferation (31), whereas moderate concentrations of H_2O_2 induce growth arrest and high concentrations induce apoptosis and/or necrosis (6). Enzymes that are involved in oxidant generation or oxidant scavenging are also critically involved in the regulation of cell growth (16, 18, 21).

Increasing evidence suggests that ROS and RNS participate in the regulation of cell-cycle control and cell proliferation. ROS play an important role in cell growth mediated by peptide growth factors and cytokines, including PDGF (31), vascular endothelial growth factor, insulin, tumor necrosis factor, and angiotensin II (8). On the other hand, higher levels of ROS and RNS associated with oxidative stress cause growth arrest or apoptosis. The pathways that regulate cell-cycle progression and induction of apoptosis are remarkably similar and are closely interconnected by key regulatory molecules and signal transduction cascades (32). A more comprehensive review of the signaling cascades involved in ROS-mediated cell-cycle control is included in this *Forum* (5). A greater understanding of these mechanisms by which oxidative stress controls these pathways should help to identify targets for therapeutic intervention.

The process of cell-cycle transition can be divided temporally into the G_1 phase, the S phase in which DNA synthesis takes place, the G_2 phase, and mitosis (M phase). Quiescent cells are found in the G_0 phase and exhibit minimal mRNA and protein synthesis. Upon stimulation, quiescent cells enter the G_1 phase, allowing for the synthesis of mRNA and proteins necessary for DNA synthesis in the ensuing S phase. During the G_2 phase, the cell synthesizes additional mRNAs in preparation for mitosis. There are two primary checkpoints that regulate cell-cycle progression. The G_1/S checkpoint prevents the replication of damaged DNA and is regulated by a balance between growth-stimulating and growth-inhibitory responses. The G_1/S and G_2/M checkpoints control the sequence and timing of cell-cycle transitions, allowing for the integration of cell division with environmental stimuli and the monitoring of DNA damage to maintain genomic integrity. An additional spindle assembly checkpoint functions to delay mitosis until the mitotic spindle is correctly formed (25). The regulation of G_1 progression and G_1/S transition is governed by a delicate interplay of cyclins, cyclin-dependent kinases (Cdks), and their inhibitors. Cyclin D1 and E are rapidly synthesized during G_1 and bind to CdK4 and CdK2, respectively. $p21^{WAF1}$ and $p27^{Kip1}$ are important negative regulators of cyclin/Cdk interactions. $p21^{WAF}$ is up-regulated by p53 and inhibits Cdk4/6 activity. $p27^{Kip1}$ inhibits CdK2 activity, thus preventing the phosphorylation of transcription factors critical for entry into the S phase (17). A variety of other gene products can interact with and modulate activities of the cyclin/Cdk complexes. Proliferating cell nuclear antigen, a cofactor for DNA polymerase δ, is negatively regulated by $p21^{WAF1}$ (12). The retinoblastoma protein (Rb) is a major target of Cdk4/6. Upon phosphorylation, Rb dissociates from the E2F transcription factor, enabling E2F to initiate gene transcription. On the other hand, transition through the G_2/M checkpoint requires complex formation between Cdc2 (cell division 2 kinase) and cyclin B to form the "mitosis-promoting factor." Cdc2 activation requires phosphorylation on Thr^{161} and dephosphorylation of Tyr^{15} by Cdc25 phosphatase (23).

ROS and RNS exert diverse effects on cell-cycle progression. In general, the growth-promoting actions of these species appear to be mediated through the activation of kinase signaling cascades, redox regulation of tyrosine phosphatases, and

[1]Laboratory of Cardiac Energetics, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD.
[2]Department of Pharmacology and Experimental Therapeutics, Louisiana State University Health Sciences Center, New Orleans, LA.

direct activation of transcription factors. Low concentrations of H_2O_2 increase cell proliferation via increased cyclin D expression and G_1/S transition in fibroblasts (19) and via decreased expression of $p27^{Kip1}$ in prostate tumor spheroids (33). H_2O_2 also mediates cell growth and cell-cycle regulation caused by targeted overexpression of the Nox1 subunit of the NADPH oxidase (1). The ability of ROS to promote G_1/S transition is further supported by the finding that antioxidant treatment often blocks proliferation and cell-cycle progression. For example, the antioxidant *N*-acetyl-L-cysteine induces G_1 arrest by decreasing cyclin D1, increasing $p27^{Kip1}$ protein levels and hypophosphorylation of Rb (20). This topic will be more fully addressed in this issue (4, 21, 24). Finally, redox responsiveness of transcription factors such as nuclear factor-κB regulates the expression of cell-cycle proteins to promote proliferation (13).

Growth arrest associated with higher, sustained levels of ROS and RNS appears to involve the induction of inhibitory cell-cycle control proteins, especially p21, and repression of cyclins (3). Transient cell-cycle arrest in G_1/S protects against oxidant-induced DNA damage and apoptosis (27). On the other hand, sustained production of ROS and RNS may actually prevent cell-cycle reentry (34) and lead to cellular senescence (8). More recently, the redox-sensitive family of Forkhead box class O (FOXO) transcription factors has been shown to promote cell-cycle arrest in G_1 by transcriptional up-regulation of the Cdk inhibitor $p27^{Kip1}$ and down-regulation of cyclin D1 (7).

A role for oxidative stress in the control of the G_2/M checkpoint has only recently been documented. Cdc25 phosphatase controls the activation of Cdc2/cyclin B protein kinase and entry into mitosis in eukaryotic cells. H_2O_2 causes oxidation of a reactive-site cysteine residue in Cdc25 tyrosine phosphatase that causes subsequent binding to 14-3-3 proteins, nuclear export, and degradation (28–30). As Cdc25-mediated dephosphorylation of Tyr^{15} of Cdc2 is required for its activation, this oxidant-induced decrease in nuclear Cdc25 content would lead to cell-cycle arrest at G_2/M. Oxidative stress also promotes nuclear export of Cdc25B via protein kinase B/Akt-dependent phosphorylation on Ser^{353} (2). The FOXO transcription factor AFX (acute lymphocytic leukemia-1 fused gene from chromosome X) also participates in oxidant-induced G_2/M arrest through induction of GADD45 (growth arrest- and DNA damage-inducible protein) that is implicated in G_2/M arrest and DNA repair (10, 11). Although the signaling cascades that regulate oxidative stress-induced G_2/M arrest have not been fully elucidated, recent studies have implicated protein kinase B/Akt and the tyrosine kinase Syk (2, 14).

Much less is known about the redox control of the spindle assembly checkpoint. A recent report suggests that mitotic spindle pole formation is an important component of H_2O_2-induced mitosis in type II pneumocytes (26). Several mitotic protein kinases, such as Cdc2, have been implicated in spindle regulation (22), suggesting a possible role of redox control of Cdc2 activation by Cdc25C in the spindle checkpoint. Finally, both the p38 and ERK1/2 (15) mitogen-activated protein kinase pathways, which are regulated by ROS, appear to play a crucial role in mitotic spindle assembly. Further investigation is required to define a role for oxidative stress in the regulation of the spindle assembly checkpoint.

Understanding the molecular and cellular mechanisms by which ROS and RNS regulate cell-cycle progression is an important research topic in cell biology and pathophysiology with important implications for cancer, aging, cardiovascular and renal disease, and pulmonary and neuronal injury. This *Forum* on redox control of the cell cycle includes both reviews and original contributions that significantly expand our current understanding of how an intricate balance between oxidant and antioxidant mechanisms regulates cell proliferation, senescence, and apoptosis.

ABBREVIATIONS

Cdc2, cell division 2 kinase; Cdk, cyclin-dependent kinase; FOXO, Forkhead box class O; H_2O_2, hydrogen peroxide; PDGF, platelet-derived growth factor; Rb, retinoblastoma protein; RNS, reactive nitrogen species; ROS, reactive oxygen species.

REFERENCES

1. Arnold RS, Shi J, Murad E, Whalen AM, Sun CQ, Polavarapu R, Parthasarathy S, Petros JA, and Lambeth JD. Hydrogen peroxide mediates the cell growth and transformation caused by the mitogenic oxidase Nox1. *Proc Natl Acad Sci, U S A* 98: 5550–5555, 2001.
2. Baldin V, Theis-Febvre N, Benne C, Froment C, Cazales M, Burlet-Schiltz O, and Ducommun B. PKB/Akt phosphorylates the CDC25B phosphatase and regulates its intracellular localisation. *Biol Cell* 95: 547–554, 2003.
3. Barnouin K, Dubuisson ML, Child ES, Fernandez de Mattos S, Glassford J, Medema RH, Mann DJ, and Lam EW. H_2O_2 induces a transient multi-phase cell cycle arrest in mouse fibroblasts through modulating cyclin D and p21Cip1 expression. *J Biol Chem* 277: 13761–13770, 2002.
4. Burch PM and Heintz NH. Redox regulation of cell-cycle reentry: cyclin D1 as a primary target for the mitogenic effects of reactive oxygen and nitrogen species. *Antioxid Redox Signal* 7: 741–751, 2005.
5. Cakir Y and Ballinger SW. Reactive species-mediated regulation of cell signaling and the cell cycle: the role of MAPK. *Antioxid Redox Signal* 7: 726–740, 2005.
6. Deshpande NN, Sorescu D, Seshiah P, Ushio-Fukai M, Akers M, Yin Q, and Griendling KK. Mechanism of hydrogen peroxide-induced cell cycle arrest in vascular smooth muscle. *Antioxid Redox Signal* 4: 845–854, 2002.
7. Dijkers PF, Medema RH, Pals C, Banerji L, Thomas NS, Lam EW, Burgering BM, Raaijmakers JA, Lammers JW, Koenderman L, and Coffer PJ. Forkhead transcription factor FKHR-L1 modulates cytokine-dependent transcriptional regulation of p27(KIP1). *Mol Cell Biol* 20: 9138–9148, 2000.
8. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 82: 47–95, 2002.
9. Finkel T. Oxidant signals and oxidative stress. *Curr Opin Cell Biol* 15: 247–254, 2003.
10. Furukawa-Hibi Y, Yoshida-Araki K, Ohta T, Ikeda K, and Motoyama N. FOXO forkhead transcription factors induce

G_2-M checkpoint in response to oxidative stress. *J Biol Chem* 277: 26729–26732, 2002.
11. Furukawa-Hibi Y, Kobayashi Y, Chen C, and Motoyama N. FOXO transcription factors in cell-cycle regulation and the response to oxidative stress. *Antioxid Redox Signal* 7: 752–760, 2005.
12. Gibbs E, Kelman Z, Gulbis JM, O'Donnell M, Kuriyan J, Burgers PMJ, and Hurwitz J. The influence of the proliferating cell nuclear antigen-interacting domain of p21CIP1 on DNA synthesis catalyzed by the human and *Saccharomyces cerevisiae* polymerase delta holoenzymes. *J Biol Chem* 272: 2373–2381, 1997.
13. Haddad JJ. Oxygen sensing and oxidant/redox-related pathways. *Biochem Biophys Res Commun* 316: 969–977, 2004.
14. He J, Takano T, Ding J, Gao S, Noda C, Sada K, Yanagi S, and Yamamura H. Syk is required for p38 activation and G2/M arrest in B cells exposed to oxidative stress. *Antioxid Redox Signal* 4: 509–515, 2002.
15. Horne MM and Guadagno TM. A requirement for MAP kinase in the assembly and maintenance of the mitotic spindle. *J Cell Biol* 161: 1021–1028, 2003.
16. Immenschuh S and Baumgart-Vogt E. Peroxiredoxins, oxidative stress, and cell proliferation. *Antioxid Redox Signal* 7: 768–777, 2005.
17. Li JM and Brooks G. Cell cycle regulatory molecules (cyclins, cyclin-dependent kinases and cyclin-dependent kinase inhibitors) and the cardiovascular system; potential targets for therapy? *Eur Heart J* 20: 406–420, 1999.
18. Li Volti G, Sacerdoti D, Sangras B, Vanella A, Alexandre M, Scapagnini G, Falck JR, and Abraham NG. Carbon monoxide signaling in promoting angiogenesis in human microvessel endothelial cells. *Antioxid Redox Signal* 7: 704–710, 2005.
19. Martinez-Munoz C, Post JA, Verkleij AJ, Verrips CT, and Boonstra J. The effect of hydrogen peroxide on the cyclin D expression in fibroblasts. *Cell Mol Life Sci* 58: 990–996, 2001.
20. Menon SG, Sarsour EH, Spitz DR, Higashikubo R, Sturm M, Zhang H, and Goswami PC. Redox regulation of the G1 to S phase transition in the mouse embryo fibroblast cell cycle. *Cancer Res* 63: 2109–2117, 2003.
21. Menon SG, Coleman MC, Walsh SA, Spitz DR, and Goswami PC. Differential susceptibility of nonmalignant human breast epithelial cells and breast cancer cells to thiol antioxidant-induced G_1 delay. *Antioxid Redox Signal* 7: 711–718, 2005.
22. Nigg EA. Mitotic kinases as regulators of cell division and its checkpoints. *Nat Rev Mol Cell Biol* 2: 21–32, 2001.
23. O'Connell MJ, Walworth NC, and Carr AM. The G2-phase DNA-damage checkpoint. *Trends Cell Biol* 10: 296–303, 2000.
24. O'Reilly MA, Vitiello PF, Gehen SC, and Staversky RJ. $p21^{Cip1/WAF1/Sdi1}$ does not affect expression of base excision DNA repair enzymes during chronic oxidative stress. *Antioxid Redox Signal* 7: 719–725, 2005.
25. Pearce AK and Humphrey TC. Integrating stress-response and cell-cycle checkpoint pathways. *Trends Cell Biol* 11: 426–433, 2001.
26. Radisavljevic ZM, Gonzalez-Flecha B, and Manasija-Radisavljevic Z. Signaling through Cdk2, importin-alpha and NuMA is required for H_2O_2-induced mitosis in primary type II pneumocytes. *Biochim Biophys Acta* 1640: 163–170, 2003.
27. Rancourt RC, Hayes DD, Chess PR, Keng PC, and O'Reilly MA. Growth arrest in G1 protects against oxygen-induced DNA damage and cell death. *J Cell Physiol* 193: 26–36, 2002.
28. Rudolph J. Catalytic mechanism of Cdc25. *Biochemistry* 41: 14613–14623, 2002.
29. Rudolph J. Redox regulation of the Cdc25 phosphatases. *Antioxid Redox Signal* 7: 761–767, 2005.
30. Savitsky PA and Finkel T. Redox regulation of Cdc25C. *J Biol Chem* 277: 20535–20540, 2002.
31. Sundaresan M, Yu Z-X, Ferrans VJ, Irani K, and Finkel T. Requirement for generation of HO for platelet-derived growth factor signal transduction. *Science* 270: 266–269, 1995.
32. Tessema M, Lehmann U, and Kreipe H. Cell cycle and no end. *Virchows Arch* 444: 313–323, 2004.
33. Wartenberg M, Diedershagen H, Hescheler J, and Sauer H. Growth stimulation versus induction of cell quiescence by hydrogen peroxide in prostate tumor spheroids is encoded by the duration of the Ca^{2+} response. *J Biol Chem* 274: 27759–27767, 1999.
34. Yuan Z, Schellekens H, Warner L, Janssen-Heininger Y, Burch P, and Heintz NH. Reactive nitrogen species block cell cycle re-entry through sustained production of hydrogen peroxide. *Am J Respir Cell Mol Biol* 28: 705–712, 2003.

Address reprint requests to:
Pamela A. Lucchesi, Ph.D.
Department of Pharmacology
Louisiana State University Health Sciences Center
1901 Perdido Street–P7-1
New Orleans, LA 70112

E-mail: plucch@lsuhsc.edu

This article has been cited by:

1. Kailash Prasad. 2011. Do Statins have a Role in Reduction/Prevention of Post-PCI Restenosis?. *Cardiovascular Therapeutics* no-no. [CrossRef]
2. Li Zhang, Olivia R. Sheppard, Ajay M. Shah, Alison C. Brewer. 2008. Positive regulation of the NADPH oxidase NOX4 promoter in vascular smooth muscle cells by E2F. *Free Radical Biology and Medicine* **45**:5, 679-685. [CrossRef]
3. V. Sitaramam, R. Bhate, P. Kamalraj, S. Pachapurkar. 2008. Respiration hastens maturation and lowers yield in rice. *Physiology and Molecular Biology of Plants* **14**:3, 253-271. [CrossRef]
4. Jianfei Jiang, Natalia A. Belikova, Adam T. Hoye, Qing Zhao, Michael W. Epperly, Joel S. Greenberger, Peter Wipf, Valerian E. Kagan. 2008. A Mitochondria-Targeted Nitroxide/Hemigramicidin S Conjugate Protects Mouse Embryonic Cells Against Gamma Irradiation. *International Journal of Radiation Oncology*Biology*Physics* **70**:3, 816-825. [CrossRef]