Cell Cycle 11:18, 3339-3340; September 15, 2012; © 2012 Landes Bioscience

A novel G_1 checkpoint mediated by the p57 CDK inhibitor and p38 SAPK promotes cell survival upon stress

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Eukaryotic cells have developed sophisticated devices to constantly monitor changes in the extracellular environment and to orchestrate a proper cellular response. Among the most conserved signaling modules throughout evolution are the mitogen-activated, protein kinase (MAPK) cascades. A prototypical family of MAPKs that respond to stress is the p38 stress-activated protein kinase (SAPK). In mammals, p38 SAPKs are activated by external stimuli, such as cytokines, infection, oxidative stress, heat shock, DNA damage and osmostress, and regulate biological processes such as immune response, inflammation, development, proliferation and survival.[1] Regardless, the sensor mechanism engaged, signaling converges on p38, which will mediate an adaptive response to stress. p38 has been regarded to act as a tumor suppressor capable of controlling cell proliferation through the activation of different cell cycle checkpoints. Upon DNA damage, p38 mediates a G_2/M checkpoint involving the downstream kinase MK2. MK2 phosphorylates Cdc25, which creates a docking site for 14-3-3 proteins that will retain Cdc25 into the cell cytoplasm, thus preventing Cdc2-cyclin B dephosphorylation and activation.[2,3] Genotoxic stresses also contribute to G_1/S arrest through the stabilization of the CDK inhibitors $p21^{Cip1}$ and $p27^{Kip1}$.[4,5] Despite the p38 relevance on cell cycle control, the mechanisms by which this SAPK regulate cell cycle are not yet fully understood.

A recent study has shown that exposing mammalian cells to osmostress resulted in a strong G_1 delay, suggesting the existence of an osmostress-regulated checkpoint.[6] $p38^{-/-}$ cells were not able to arrest properly, indicating that this G_1 checkpoint relied on p38. Cell cycle progression throughout G_1 is controlled by G_1 cyclin-dependent kinases (CDKs), which activity is, in turn, regulated by several CDK inhibitors (CDKis).[7] In yeast, G_1 progression upon osmostress by the p38/Hog1 SAPK is exerted by downregulating cyclin expression and the direct targeting of the Sic1 CDKi.[8,9] In mammals, osmostress downregulated Cdk2-CyclinA/E activity in a p38-dependent manner. Therefore, p38 might be regulating CDK2 activity through the targeting of CDKis. p38 was able to directly phosphorylate the CDKi $p57^{Kip2}$ at T143 both in vitro and in vivo. This particular site is located in a central region of p57 not shared by p21 or p27. Cells genetically lacking $p57^{Kip2}$ failed to properly arrest at G_1 upon osmostress. Osmostress-induced $p57^{Kip2}$ phosphorylation neither affected its protein half-life nor its nuclear localization. Instead, $p57^{Kip2}$ phosphorylation at T143 increased its protein affinity toward cyclin A/Cdk2 complexes in vitro in contrast to a non-phosphorylable $p57^{Kip2}$ mutant (T143A). Altogether, CDK2 activity might be differentially regulated upon $p57^{Kip2}$ phosphorylation. Indeed, $p57^{Kip2}$ inhibited Cdk2-cyclin A activity in a dose-dependent manner, and this was further increased upon phosphorylation at T143. The reintroduction of wild-type $p57^{Kip2}$ into $p57^{-/-}$ cells fully rescued the osmostress-induced G_1 checkpoint and reestablished in vivo CDK2 activity regulation. Importantly, the T143A mutant was unable to fully restore the osmostress-induced G_1 checkpoint and failed to regulate in vivo CDK2 activity. Therefore, p38 is regulating a novel G_1 checkpoint by directly targeting the CDKi $p57^{Kip2}$ (**Fig. 1**). Since p38 responds to a number of environmental insults, it was possible that such a G_1 checkpoint might be important for proper cell adaptation to general stresses. Thus, $p38^{-/-}$ and $p57^{-/-}$ cells underwent programmed cell death after the exposure to different insults such as osmostic shock, oxidative stress and ionomycin. Notably $p57^{-/-}$ cell viability was rescued by reintroducing wild-type p57, but not by the T143A mutant, pointing out the biological relevance of this novel phosphorylation site to arrest cell cycle in response to stress.

Surprisingly, neither p21 nor p27 compensated for the lack of p57. Although p21 was found to be upregulated in the absence of p57, it was unable to regulate the G_1 phase and cell viability upon stress. Additionally, $p27^{-/-}$ cell viability was not compromised upon cell stress, indicating that p57 is just enough to integrate a broad range of cell stresses in a p38-dependent manner. Both p21 and p27 are p38 targets. Then, why do these CDKs not contribute to cell survival? One explanation would be the type of stress. UV and DNA damaging agents activate the p53, ATM/ATR as well as the p38 SAPK pathways, resulting in the regulation of both p21 and p27. In that scenario, p57 is not relevant, since UV-induced DNA damage did not affect $p57^{-/-}$ viability but strongly compromised $p38^{-/-}$ cell fitness. Also, downregulation of p38 substrates such as Cdt1 and MK2, which are involved in controlling S-phase and G_2/M checkpoints, respectively, do not contribute to cell survival upon stress.[2,3,10]

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Submitted: 06/27/12; Accepted: 06/29/12
http://dx.doi.org/10.4161/cc.21840
Comment on: Joaquin M, et al. EMBO J 2012; 31:2952-64; PMID:22569127; http://dx.doi.org/10.1038/emboj.2012.122.

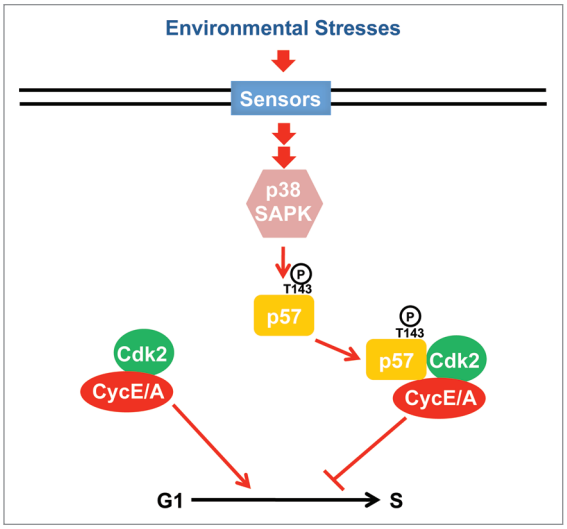

Figure 1. Schematic diagram depicting the mechanism by which p38 SAPK activation promotes G_1 cell cycle arrest through $p57^{Kip2}$ phosphorylation. Transition from G_1 to S phase requires Cdk2-cyclinE/A activity. Upon an environmental stress, p38 SAPK becomes transiently activated and phosphorylates the T143 of the CDKi $p57^{Kip2}$. p57 phosphorylation increases its affinity toward Cdk2-cyclin E/A complexes, which become inactivated and impose a G_1 delay. Activation of this G_1 checkpoint is essential for proper cell adaptation and survival to stress.

Altogether, p38 and p57 impose a G_1 cell cycle arrest to promote cell survival upon cell stresses. But why is so important to delay G_1 cell cycle progression? We may speculate that to build up a proper cell adaptation response, cells need time to upregulate a specific set of genes. Indeed, stress-induced p38 controls the expression of many genes involved in cell proliferation as well as cell survival in a time-dependent manner.[11] The relationship between cell cycle control and gene expression regulation has not been studied thoroughly, but delaying G_1 progression might be a step necessary to upregulate the essential genes needed for cell adaptation before progressing into S phase.

References

1. Cuadrado A, et al. Biochem J 2010; 429:403-17; PMID:20626350; http://dx.doi.org/10.1042/BJ20100323.
2. Manke IA, et al. Mol Cell 2005; 17:37-48; PMID:15629715; http://dx.doi.org/10.1016/j.molcel.2004.11.021.
3. Reinhardt HC, et al. Cancer Cell 2007; 11:175-89; PMID:17292828; http://dx.doi.org/10.1016/j.ccr.2006.11.024.
4. Cuadrado M, et al. Cancer Res 2009; 69:8726-32; PMID:19843869; http://dx.doi.org/10.1158/0008-5472.CAN-09-0729.
5. Lafarga V, et al. Mol Cell Biol 2009; 29:4341-51; PMID:19528229; http://dx.doi.org/10.1128/MCB.00210-09.
6. Joaquin M, et al. EMBO J 2012; 31:2952-64; PMID:22569127; http://dx.doi.org/10.1038/emboj.2012.122.
7. Besson A, et al. Dev Cell 2008; 14:159-69; PMID:18267085; http://dx.doi.org/10.1016/j.devcel.2008.01.013.
8. Escoté X, et al. Nat Cell Biol 2004; 6:997-1002; PMID:15448699; http://dx.doi.org/10.1038/ncb1174.
9. Adrover MÀ, et al. Sci Signal 2011; 4:ra63; PMID:21954289; http://dx.doi.org/10.1126/scisignal.2002204.
10. Chandrasekaran S, et al. Mol Cell Biol 2011; 31:4405-16; PMID:21930785; http://dx.doi.org/10.1128/MCB.06163-11.
11. Ferreiro I, et al. BMC Genomics 2010; 11:144; PMID:20187982; http://dx.doi.org/10.1186/1471-2164-11-144.