

## DNA Damage Signaling Recruits the RNA Polymerase II Binding Protein Che-1 to the p53 Promoter

New work by Bruno et al. (2006) describes a mechanistic switch of the proliferation-promoting Che-1 activator of E2F-target genes into a cell-cycle inhibitor in response to DNA damage, through Che-1 relocalization to, and activation of, the p53 tumor suppressor gene.

In response to genotoxic insults, mammalian cells activate a complex network of proteins, the key elements of which are the checkpoint signaling kinases ATM and ATR and their effector kinases Chk2 and Chk1, respectively (Kastan and Bartek, 2004). One of the key substrates of the ATM/ATR and Chk2/Chk1 kinases is the p53 tumor suppressor protein that, once posttranslationally modified, activates transcription of genes that induce cell-cycle arrest, DNA repair, or apoptosis and represses transcription of genes that promote cell-cycle progression (Toledo and Wahl, 2006; Vousden and Lu, 2002).

Initially, the regulation of p53 by checkpoint signaling was thought to be almost exclusively at the posttranscriptional level, with the checkpoint kinases stabilizing the otherwise short-lived p53 protein by enhancing its transcriptional activity (Toledo and Wahl, 2006; Vousden and Lu, 2002). More recently, p53 turned out to be regulated also at the transcriptional (Wang and El-Deiry, 2006) and translational (Takagi et al., 2005) levels. Now, a study by Bruno et al. (2006) published in the December

issue of *Cancer Cell* identifies a novel pathway, involving Che-1, by which checkpoint kinases regulate p53.

Che-1 is an RNA polymerase II binding protein involved in the transcription of E2F-target genes. Che-1 was previously shown to interact with the retinoblastoma protein (Rb) and inhibit its ability to suppress expression of E2F, pointing to a proproliferative role (Fanciulli et al., 2000). However, more recently, Che-1 was shown to have antiproliferative activity by inducing expression of p21Waf1 (Di Padova et al., 2003). To follow up on this last observation, Bruno et al. (2006) examined whether Che-1 was involved in the DNA damage response. Doxorubicin and other DNA damaging agents led to stabilization of the Che-1 protein, an effect mediated by the kinases ATM and Chk2. The former kinase phosphorylated Che-1 on Ser187, whereas the latter targeted residues Ser141, Ser474, and Ser508 (Figure 1). Substitution of these four serines with alanine completely abrogated stabilization of Che-1 after DNA damage.

Because Che-1 is an RNA polymerase II binding protein, Bruno et al. (2006) subsequently examined whether stabilization of Che-1 after DNA damage led to increased occupancy at specific promoters. Indeed, Che-1 localized to the promoters of the p53 and p21Waf1 genes after DNA damage, and this was associated with increased transcription of these two genes (Figure 1). Increased transcription of both the p53 and p21Waf1 genes contributed to the increase in p53 and p21 protein levels after DNA damage, as evidenced by decreased p53/p21 protein accumulation in cells in which Che-1 was depleted by siRNA.

At a phenotypic level, Che-1 activation by the DNA damage checkpoint was critical for maintenance of G2 arrest after diverse genotoxic insults. Cells in which

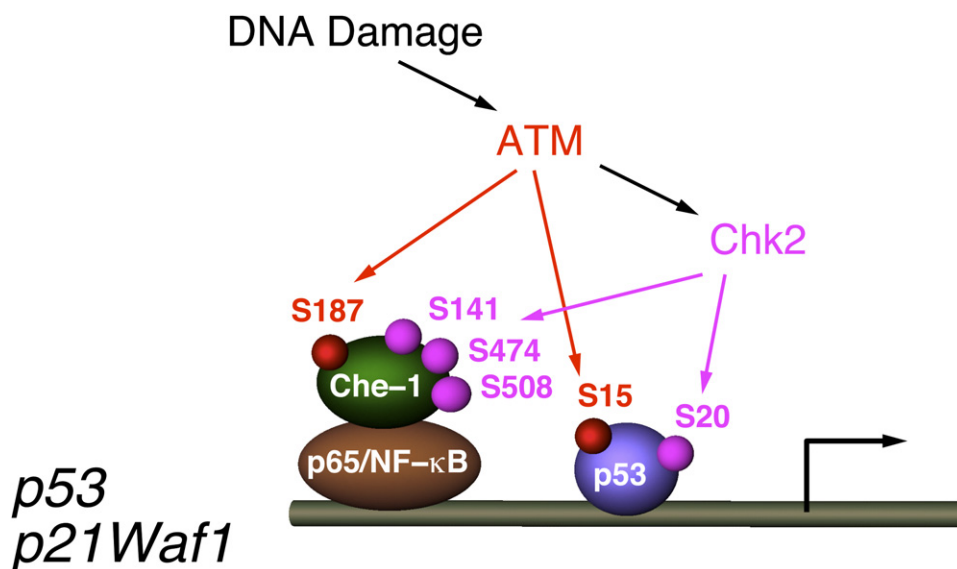


Figure 1. Regulation of p53 Target Genes by p53 and Che-1 after DNA Damage

The checkpoint kinases ATM and Chk2 phosphorylate and activate both the p53 and Che-1 proteins, which are then recruited (Che-1 through its interaction with the p65 subunit of the NF-κB transcription factor) to the promoters of the p53 gene and p53-target genes, such as p21Waf1, to activate transcription. See text for more details.

Che-1 was depleted arrested in G2 after DNA damage, but only for about 12 hr, and then entered mitosis, whereas control cells remained arrested in G2 even 24 hr after being irradiated. Maintenance of the G2 checkpoint arrest by Che-1 was p53 dependent and apparently involved increased expression of *p21Waf1* and increased repression of *Cdc25C*, two p53 target genes that are key mediators of the p53-dependent G2 arrest (Bruno et al., 2006; Kastan and Bartek, 2004; Vousden and Lu, 2002). In addition, Bruno et al. show that Che-1 depletion sensitized human cancer cells to treatment with DNA damaging anticancer drugs, suggesting that Che-1 may represent a possible target for drug discovery.

Although Bruno et al. (2006) have not examined any potential role of the Che-1-mediated control of p53/p21 in the G1 checkpoint, such a function is very likely, given the established role of the p53-p21 pathway in the long-term G1 arrest and establishment of cellular senescence (Kastan and Bartek, 2004; Toledo and Wahl, 2006; Vousden and Lu, 2002). In light of the recent evidence for a causal relationship between DNA damage signaling from ATM/ATR and Chk2/Chk1 kinases and oncogene-induced senescence (Bartkova et al., 2006; Di Micco et al., 2006), it is tempting to speculate that the new Che-1/p53/p21 pathway reported by Bruno et al. (2006) contributes to the anticancer barrier in premalignant human tumors provided by the DNA damage checkpoints (Bartkova et al., 2006; Di Micco et al., 2006).

The findings described by Bruno et al. (2006) are consistent with a recent report showing increased transcription of the *p53* gene after DNA damage (Wang and El-Deiry, 2006). Wang and El-Deiry attributed the increased transcription of the *p53* gene to a positive feedback loop and identified a p53 binding site in the *p53* gene, whereas Bruno et al. attribute the increased transcription of the *p53* gene after DNA damage to binding of Che-1 to the *p53* promoter (Wang and El-Deiry, 2006; Bruno et al., 2006). It is plausible that the p53 and Che-1 proteins, both targets of the same DNA damage checkpoint kinases, cooperate to induce expression of *p53* and p53-target genes (Figure 1). By enlisting two transcriptional activators, each one of which can be induced by DNA damage, the cell may be able to achieve robust activation of gene transcription in response to DNA damage.

The emerging complexity of the regulatory networks that converge to modulate p53 and its targets in mammalian cells is already bewildering (Toledo and Wahl, 2006; Vousden and Lu, 2002). The new insight into this sophisticated molecular machinery, provided by Bruno et al. (2006), brings, however, more than just a simple addition of another upstream regulator of p53. Conceptually, this new mechanism provides an example of an elegant molecular switch, in that Che-1 and its regulation of the Rb/E2F interplay promotes progression

through unperturbed cell cycles, whereas the ATM/ATR-Chk2-phosphorylated Che-1 under DNA damage conditions becomes relocated to *p53* and *p21Waf1* promoters to turn into a cell-cycle-inhibitory element. Viewed from a broader perspective of cell physiology and tumorigenesis, Che-1 seems to belong to a class of important regulators of cell growth whose properties meet the criteria for both the candidate proto-oncogene and tumor suppressor. Future research in this area promises to shed more light on this potential dual role of Che-1 and no doubt reveal further surprises.

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#### Selected Reading

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