The Regulation of p53 Protein Function by Phosphorylation

Nicola J. Maclaine and Theodore Hupp*

Introduction

p53 is a sequence-specific DNA-binding protein and stress-activated transcription factor that controls the expression of hundreds of genes implicated in a variety of physiological responses to genome instability, virus infection and interferon production, DNA damage, and metabolic stresses. The vast numbers of gene products mediating the p53 signal coordinate many repair processes, some of which include elimination of damaged proteins, DNA repair, ATP generation via oxidative phosphorylation, organelar functions that maintain autophagy signaling and mitochondrial function, the cell division cycle, and programmed cell death. The implications of this stress-induced transcription reprogramming by p53 is that cell and tissue integrity can be maintained, thereby contributing to organism health and viability.

Inactivating missense mutations in p53 are very common in a wide range of human cancers, indicating a critical role for p53 as a cancer suppressor in very distinct tissue microenvironments. These mutations reside predominantly in the core DNA-binding domain (Fig. 1), and result in a p53 protein with an altered conformation and reduced sequence-specific DNA-binding function. These mutations suppress p53 transcription, reduce the cellular repair capacity, and stimulate cancer development. As p53 is a conformationally flexible and thermodynamically unstable protein, biophysical studies have suggested there is promise in drug developments aimed at stabilizing the mutant p53 conformation into a wild-type state, and reengaging the p53 dependent transcription.

Transgenic technologies in mice have supported biochemical and clinical data showing a critical role for the DNA-binding function of p53 in cancer suppression. Animals null for p53 strikingly develop cancer at an advanced rate. By contrast, deletion of many of the p53-inducible genes do not give the same tumor incidence or tumor spectrum as p53-null animals, further highlighting the role of p53 itself as a central hub in the integration of tissue repair triggers. There is one intriguing exception: animals double null for ataxia telangiectasia mutated (ATM) and the p53-inducible gene p21 have a similar tumor spectrum and death incidence to the p53-null animals. This suggests that ATM and p21 form a positive genetic circuit in the p53-dependent cancer suppression mechanism.

*p53 Regulation

p53 protein function is regulated post-translationally by coordinated interaction with signaling proteins including protein kinases, acetyltransferases, methyltransferases, and ubiquitin-like modifying enzymes (Fig. 1). The majority of the sites of covalent modification occur at intrin-
p53 is known to be a transcriptional regulator that plays a crucial role in controlling cell cycle progression, apoptosis, and genome integrity. Its activity is regulated by various mechanisms, including post-translational modifications such as phosphorylation, acetylation, ubiquitination, methylation, neddylation, and sumoylation.

**Figure 1. Sites of post-translational modifications on p53.** The 393 amino acid domain structure of human p53 is depicted showing the sites of post-translational modification including phosphorylation, acetylation, ubiquitination, methylation, neddylation, and sumoylation. Abbreviations: N-terminal transactivation domain (TAD); proline-rich domain (PRD); tetramerization domain (TET); C-terminal regulatory domain (REG); arginine (R); lysine (K); serine (S); threonine (T).

**Figure 2. Linear peptide docking sites in p53.** A) Linear peptide docking sites for enzymes that regulate p53 function. The N-terminus is composed of three transactivation motifs, TAD1, TAD2, and Proline-repeat domain (PRD). A key regulatory domain in the C-terminus (REG) contains the acetylation motifs and phosphorylation site and flanks the Tetramerization domain (TET). The overlapping, but distinct, linear polypeptide docking motifs for p53 regulators include the acetyltransferase p300, the E3 ubiquitin ligase MDM2, iASPP, and the protein kinases including CDK, CK2, CK1, and CHK2 are highlighted. B) Conservation of key phospho-acceptor sites between urochordate and human. The panel highlights the conservation of amino acids and phospho-acceptor sites in the BOX-I transactivation domain of p53 (TAD1 in (A)) between human and urochordate (Ciona intestinalis). The ATM phospho-acceptor site at Ser15 and the Calcium Calmodulin kinase/CK1 phospho-acceptors sites at Thr18 and Ser20 are highlighted as indicated.