

Extra Views

p53^{QS}

An Old Mutant Teaches Us New Tricks

Thomas M. Johnson¹

Laura D. Attardi^{1,2,*}

¹Division of Radiation and Cancer Biology; Department of Radiation Oncology; and
²Department of Genetics; Stanford University School of Medicine; Stanford,
California USA

*Correspondence to: Laura D. Attardi; Department of Radiation Oncology;
Stanford University School of Medicine; Stanford, California USA; Tel.:
950.725.8424; Fax: 650.723.7382; Email: attardi@stanford.edu

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ABSTRACT

The p53 protein functions as a tumor suppressor, preventing aberrant cellular proliferation in response to various genotoxic and non-genotoxic stress signals. Although p53's ability to induce apoptosis is critical to its capacity to suppress tumorigenesis, the role of transcriptional activation in p53's apoptotic function has been highly controversial. To address this issue, our laboratory generated a p53 mutant knock-in mouse strain in which residues 25 and 26, previously shown to be critical for p53's transactivation function, were mutated from leucine and tryptophan to glutamine and serine, respectively. Our analysis of cells derived from these mice provided significant insight into p53 activity at both the molecular and cellular level. In particular, our data suggest that p53 utilizes discrete mechanisms to transactivate different target genes, and that p53 employs distinct mechanisms to induce apoptosis in response to different stresses. This p53^{QS} mutant mouse strain represents a powerful means to dissect p53 function in vivo.

The p53 protein plays a critical role in preventing cancer, as demonstrated by the finding that over half of human cancers harbor mutations within the *p53* gene itself or in various components of the p53 pathway.^{1,2} p53 suppresses tumorigenesis by inducing either G₁ cell cycle arrest, cellular senescence, or apoptosis in response to stresses encountered by neoplastic cells, such as DNA damage, hypoxia, and growth factor deprivation. In particular, p53's ability to induce apoptosis has been implicated as one of the most crucial aspects of its tumor suppressor function.³ However, the means by which it induces this terminal cell fate has been extensively debated in the literature.

Initial studies of the p53 protein revealed that it possesses several features typical of a transcriptional activator, including a transactivation domain involved in interactions with the transcriptional machinery as well as a sequence specific DNA binding domain. The transactivation domain of p53 was defined in experiments showing that fusion of full length p53 to a heterologous DNA binding domain allowed for activated transcription of a reporter construct,⁴ and concomitant studies delineated the sequences sufficient for activation as residing within the first 73 amino acids of p53.⁵ The DNA binding domain was identified as a protease resistant region in the center of the protein spanning amino acids 102 through 292.⁶⁻⁸ Analysis of human tumors has shown that mutations within the *p53* gene are nearly exclusively localized within sequences encoding the DNA binding domain,⁹ and some of the most commonly mutated residues are those that mediate direct contact with the DNA.^{10,11} These epidemiological data argue that p53's ability to bind DNA in a sequence specific manner is absolutely critical for its function as a tumor suppressor.

Since p53 has been shown to possess the ability to activate transcription, a host of studies has been performed to examine the role of transcriptional activation in p53-dependent apoptosis. However, the results from these investigations have produced conflicting data as to the requirement for this function to induce cell death. While some studies utilizing transactivation-deficient p53 mutants suggested that p53-dependent target gene upregulation is critical for its ability to induce apoptosis,^{12,13} others showed that cells treated with RNA or protein synthesis inhibitors or expressing transactivation mutant forms of p53, respectively, could still undergo p53-dependent cell death.¹⁴⁻¹⁶ More recently, several groups have suggested that upon sensing a cellular stress, p53 localizes directly to the mitochondria and, acting in the capacity of a BH3-only domain protein, directly induces mitochondrial membrane permeability and apoptosis via inhibition of Bcl-2 and Bcl-x_L.^{17,18} How can these seemingly disparate results be reconciled?

To examine this issue, our laboratory generated a knock-in mouse strain, termed p53^{QS}, in which two residues in the p53 protein at codons 25 and 26 were mutated from

leucine and tryptophan to glutamine and serine, respectively.¹⁹ The corresponding mutations in human p53 (at codons 22 and 23) had previously been shown to severely compromise p53's function as a transactivator in vitro.²⁰ However, analysis of cells derived from our knock-in mice has several advantages when compared to overexpression studies in cultured cell lines. By expressing this mutant from its endogenous promoter, we obtain physiologically relevant regulation of p53 expression. Likewise, this approach allows for an evaluation of the activity of p53^{QS} in primary cells from a wide variety of tissue types, providing an opportunity to establish cell-type specific differences in downstream pathways utilized by p53. Finally, and most importantly, analysis of our knock-in mouse strain provides a means to assess the role of transactivation in tumor suppression. Intriguingly, in our initial studies examining primary mouse fibroblasts derived from these mice, we have encountered a number of surprises.¹⁹

One unanticipated finding we made is that while this molecule is significantly compromised in its ability to activate the transcription of a variety of different p53 target genes, including *p21*, *mdm2*, *noxa* and *Perp*, this apparently transactivation-deficient mutant²⁰ retained the ability to activate the transcription of certain targets such as the pro-apoptotic *bax* and *apaf-1* genes, to levels that essentially paralleled those seen with wild type p53¹⁹ (and data not shown). What do these findings reveal about the mechanism by which p53 regulates the expression of its numerous cellular targets?

In general, transcription factors need to perform at least one of several functions to induce the expression of a specific gene. One of the initial requirements is for the chromatin structure in the general vicinity of the target gene to be "opened" through post-translational modifications of histones and chromatin remodeling. This is followed by the recruitment of a wide variety of other proteins essential for transcription initiation and elongation. p53 likely stimulates multiple steps, as it has been shown to interact with histone acetyltransferases (e.g., p300/CBP), coactivators (e.g., TRAP220, TRAP80), and components of the basal transcriptional apparatus (e.g., TATA-binding protein, TAF6, TAF9). Biochemical studies have indicated that p53^{QS} retains the ability to interact with several components of the transcriptional apparatus, suggesting that other domains of p53 may be important for transactivation. For example, p53^{QS} has been shown to interact with TRAP220 as well as TBP, but not TRAP80, TAF6 or TAF9²⁰⁻³⁰ (Table 1). This idea is consistent with more recent studies demonstrating that p53 possesses a second transactivation domain (AD2) distinct from the initially defined region (AD1), in which once again, two amino acids (codons 53 and 54) are critical for the ability of p53 to activate transcription using this different surface.³¹⁻³³ These studies provide a rationale for how p53^{QS} may activate the transcription of *bax* and *apaf-1*, and potentially other target genes not yet examined (Fig. 1). It may be that the requirement for a specific activation domain depends on the particular target gene being activated, with AD1 being crucial on most genes, and AD2 being important on others. For example, p53 activation of *p21* may rely primarily on AD1 whereas p53 activation of *bax* may depend primarily on AD2. The use of distinct activation domains to transactivate different genes could relate to reliance on different cofactors according to the gene in question.

In addition to revealing different mechanisms of p53 target gene regulation, our analysis of the p53^{QS} mouse suggests that the pathway used by p53 to induce apoptosis is stress-dependent, with transcriptional activation playing a prominent role in response to certain stresses but not others. As mentioned, p53 can induce apoptosis in response to diverse stresses, including DNA damage, growth factor

Table 1 **Interactions between wild type p53 or p53^{QS} and various components of the transcriptional apparatus**

	wt p53	p53 ^{QS}	Reference
Basal Factors			
TAF 6 (TAF 70)	+	-	21
TAF 9 (TAF 31)	+	-	21, 23
TBP	+	+	20, 21, 24, 25
Histone acetyltransferases			
p300	+	-	26, 29
p300	+	+	27
CBP	+	-	28-30
CB*	+	+	27
Coactivators			
TRAP220	+	+	22
TRAP80	+	-	22

Wild type p53 has been shown to interact with multiple components of the transcriptional apparatus, whereas p53^{QS} has been shown to interact only with a subset of these proteins. Proteins are listed twice when the literature provides conflicting results as to their ability to interact with p53^{QS}.

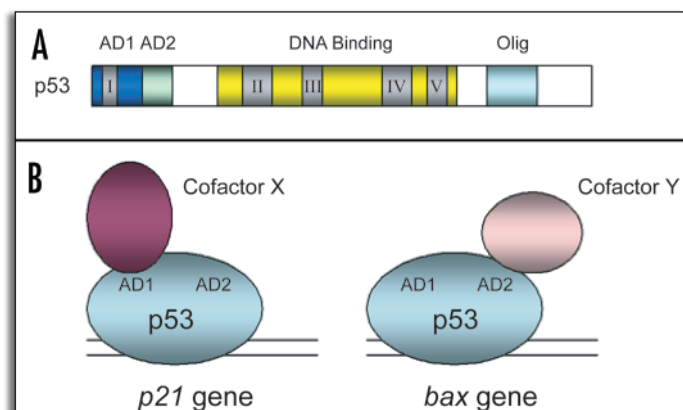


Figure 1. Description of p53 protein domains and a molecular model for p53 action at different targets. (A) The five evolutionarily conserved regions of p53 are denoted by roman numerals I-V. AD1 refers to the transactivation domain containing residues 25 and 26, and AD2 refers to the transactivation domain containing residues 53 and 54. Olig: Oligomerization domain. (B) p53 may utilize distinct transactivation surfaces at different target genes. In this model, p53 utilizes AD1 to interact with hypothetical cofactor X to upregulate expression of the *p21* gene from its responsive element in the *p21* promoter; in contrast, p53 utilizes AD2 to interact with hypothetical cofactor Y to activate *bax* gene expression from its responsive element in the *bax* first intron.

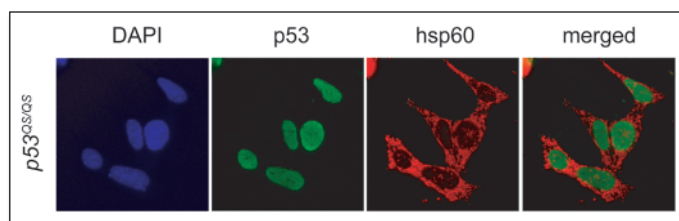


Figure 2. p53^{QS} is nuclear during hypoxia-induced apoptosis. MEFs expressing p53^{QS} and the E1A oncoprotein were placed in a low oxygen environment (< 0.02%) and analyzed after six hours, a time at which a significant number of cells are initiating apoptosis. As seen, the p53^{QS} staining colocalizes exclusively with the nuclei (marked by DAPI staining), but fails to overlap with the mitochondrial protein hsp60.

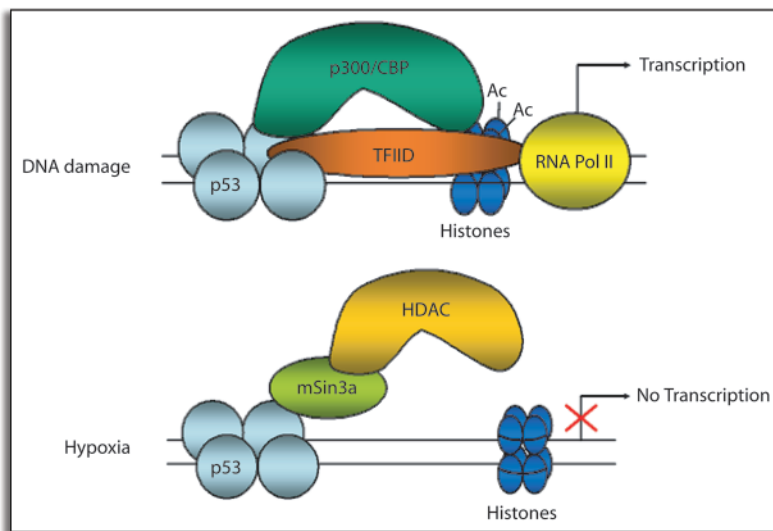


Figure 3. Model for p53 action in response to DNA damage versus hypoxia. p53 interacts with distinct components of a cell's transcriptional regulatory machinery in response to different stresses. In response to DNA damage, p53 recruits various proteins involved in the activation of basal transcription, including modifiers of chromatin structure and components of the basal transcriptional apparatus (TFIID and RNA Pol II). In contrast, in response to hypoxia, p53 recruits corepressors (mSin3a) and histone deacetylases (HDACs) to "close" chromatin structure and prevent transcription. TFIID represents a complex of TAFs and TBP.

withdrawal, or hypoxia. Interestingly, our analysis of the p53^{QS} mutant showed that it retains varying degrees of apoptotic activity, depending on the particular stress to which it is responding. Specifically, MEFs expressing the p53^{QS} protein are completely resistant to DNA damage-induced apoptosis, with levels of death paralleling those seen in cells completely lacking p53 expression, suggesting robust transactivation activity is crucial for p53 to induce apoptosis in response to this stress. However, this protein shows partial apoptotic activity in response to serum deprivation, with levels of apoptosis intermediate between wild type p53 MEFs and p53 null MEFs, suggesting p53 relies on both transactivation-dependent and -independent activities to induce death in this context. Alternatively, the threshold to induce apoptosis in response to this stress may be lower due to the cooperation of other pro-apoptotic factors induced by serum withdrawal, and thus, even the compromised capacity of p53^{QS} to activate transcription may be sufficient to explain the modest amount of death seen in MEFs expressing this p53 mutant. Perhaps most surprisingly, in response to hypoxia, this mutant maintains significant apoptotic activity, approaching that which is seen in MEFs expressing wild type p53, suggesting a transactivation-independent activity of p53 is responsible for the cell death seen in response to this stress. However, since p53^{QS} maintains significant transcriptional activity on a small percentage of the target genes we examined, it is formally possible that the transactivation of such targets is responsible for the ability of p53^{QS} to induce apoptosis in response to hypoxia. To address this issue, we examined the levels of various p53 target gene mRNAs after exposing cells to a low oxygen environment, and found that neither p53^{QS} nor wild type p53 induce the expression of any of the classical pro-apoptotic targets we evaluated. This is in stark contrast to the response of p53 to a DNA damage insult, in which the levels of various target genes increase dramatically.³⁴ The lack of clear transactivation in response to hypoxia argues against a model in which the threshold of target gene activation

sufficient to induce apoptosis is simply lower in a hypoxic cell versus a cell that has incurred DNA damage. Likewise, the rapid kinetics of hypoxia-induced apoptosis counter a model in which the gradual accumulation of p53 target gene mRNA's accounts for the cell death seen in response to this stress. While it is formally possible that the robust transactivation of a small subset of p53 target genes not examined by our laboratory or others may be responsible for apoptosis in response to hypoxia, we feel that, taken together, these exciting results suggest that these different apoptotic activities relate to distinct molecular mechanisms utilized by p53 to induce cell death.

At first glance, it seems odd that p53 would utilize different, stress-specific mechanisms to induce apoptosis; in particular, the dramatic difference between p53^{QS} apoptotic activity in response to hypoxia compared with DNA damage is intriguing. Why should a transcription factor rely so heavily on transactivation in its response to DNA damage but not hypoxia? In terms of activating an apoptotic response in a specific cellular environment, perhaps p53 is simply "playing the hand it is dealt." For example, upon treatment with a genotoxic agent such as doxorubicin, a cell is confronted with numerous DNA double stranded breaks. Depending on the tissue of origin and the cell's genetic composition, this may result in a temporary arrest in the cell cycle to repair the damage, a permanent exit from cycling known as cellular senescence, or apoptosis. Treating a cell with a DNA double strand break-inducing agent will have dramatic effects on the activity and/or expression of a wide range of proteins, but importantly, this stress has not been shown to have global effects on the cell's transcriptional and translational machinery. This is in sharp contrast to the overall transcriptional and translational effects that occur when a cell is placed in an environment lacking physiologically sufficient oxygen concentrations. In this setting, transcription is globally inhibited, a process thought to be mediated via the action of proteins such as the preinitiation complex regulator NC2.³⁵ Likewise, translation is also repressed in a hypoxic microenvironment, and this is controlled at least in part by the inhibition of the translation initiation factor eIF2 α .³⁶ As such, in an environment with critically low oxygen tension, p53 may be unable to upregulate targets such as *bax*, *nox*, *perp* and *puma* to levels sufficient to kill cells. Our data, as well as those from others,³⁷ support this idea. Although p53 has been shown to target directly to the mitochondria to induce apoptosis, recent work from our lab and others has suggested that p53^{QS} is strictly localized to the nucleus when initiating death in response to hypoxia (Fig. 2 and Hammond and Giaccia, unpublished observation). This then begs the question: what nuclear function of p53 is involved in the apoptotic response to this stress?

Previous work has suggested that p53 may be acting as a gene-specific transcriptional repressor in a hypoxic setting.³⁷ This different mode of action is supported by studies showing that p53 associates with corepressors such as mSin3a under hypoxic conditions, and that histone deacetylase inhibitors abrogate p53's ability to induce cell death in response to this stress.³⁷ Thus, instead of trying to activate the expression of pro-apoptotic targets in an environment hostile to transactivation, p53 may simply act to prevent the expression of a variety of pro-survival molecules, tipping the balance toward an apoptotic fate. Analysis of our p53^{QS}-expressing knock-in cells will be instrumental in evaluating this hypothesis. The capacity of the

p53^{QS} protein to repress transcription has been poorly defined experimentally, although it has been shown to be capable of repressing the *bcl2* gene.³⁸ Furthermore, the literature suggests that the domain required for p53-dependent recruitment of transcriptional corepressors is distinct from the transactivation domain, residing in the proline-rich region of the p53 protein.^{25,39} Thus, it is quite possible that the two mutations generated in our mice do not dramatically disrupt the overall capacity of this mutant to transrepress, accounting for the observed apoptotic activity under hypoxic conditions. Future genome-level analysis of RNA expression in hypoxic cells expressing the p53^{QS} protein will provide an ideal strategy to delineate the transrepressor capacity of this p53 mutant.

Apart from its ability to assist in understanding p53 function at the molecular level, the p53^{QS} mutant protein has enormous potential to help further understand p53's role in tumor suppression. For example, it is currently unclear which *in vivo* stresses activate p53 to prevent cancer development. This hypothetical insult could range from a DNA damage signal caused by telomere attrition or genomic instability to the hypoxic stress experienced by cells on the interior of a solid tumor to a local growth factor deficiency caused by the rapid proliferation of cells in a particular region of a tissue. Because the p53^{QS} protein exhibits a differential capacity to induce apoptosis in response to each of these stresses in oncogene-expressing MEFs, analysis of tumor development in this mouse strain will provide a powerful approach to understanding which of these insults is activating p53 *in vivo*. These studies have tremendous utility, both in understanding the molecular mechanisms important for tumor suppression, as well as in the future development of diagnostics and treatments to control cancer.

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