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The bidirectional *p53–Wrap53β* promoter is controlled by common *cis*- and *trans*-regulatory elements

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ABSTRACT

The p53 protein is an important tumor suppressor that regulates many cellular processes including maintenance of the cell cycle and apoptosis. The p53 protein maintains stability of the genome through the induction of several intracellular and extracellular factors in response to DNA damage, which in turn, stabilizes the protein, allowing it to undergo its proper mechanisms of action. We recently reported that the *p53* tumor suppressor gene has a bidirectional gene partner, *Wrap53β*. This discovery prompted the development of a bidirectional expression vector system (pLucRLuc) that is capable of measuring the output of transcripts mediated by bidirectional promoters. We have begun to study the nature of the *p53/Wrap53β* promoters. Here, we have continued these studies by incorporating mutations within the regulatory regions of the *p53/Wrap53β* bidirectional gene pair to study the effect(s) these have on the two promoters. Deletions and point mutations were created within the two promoters and their activity was examined in the pLucRLuc system. Our results demonstrated that each of the deleted sequences within the murine *p53/Wrap53β* promoters reduced the activity of each of the promoters. Co-transfections with the pLucRLuc:p53/Wrap53β bidirectional expression vector and known *p53* transcriptional regulators were also performed. Here, we demonstrate that *p53*'s bidirectional gene partner, *Wrap53β*, can also be regulated by many of the same transcription factors.

1. Introduction

The p53 protein is an important tumor suppressor that is associated with many cellular processes including, regulation of the cell cycle, DNA repair, transcriptional regulation of genes, chromosomal segregation, cell senescence, and apoptosis (Beckerman and Prives, 2010; Vogelstein et al., 2000; Zhao et al., 2009). The p53 protein has the ability to maintain stability of the genome through the induction of several intracellular and extracellular factors in response to DNA damage (Beckerman and Prives, 2010; Vousden and Lu, 2002; Zhao et al., 2009). When DNA damage occurs, p53 expression is increased, due to the increased stability of the protein. The accumulation of p53 triggers a cascade of events that can lead to either cell cycle arrest or apoptosis of the cell (Beckerman and Prives, 2010; Vousden and Lu, 2002; Zhao, et al., 2009), in turn, preventing the damaged DNA from becoming incorporated into the genome.

The expression of p53 is tightly monitored, and induced in early S-phase. This serves as a mechanism to ensure genomic stability prior to cells entering S-phase, and to ensure that the protein is rapidly induced in response to DNA damage (Takahashi et al., 2011). In addition to increased protein stability, it is generally accepted that regulation of p53 protein levels is also correlated to how the gene is transcriptionally regulated (Takaoka et al., 2003).

There is a wealth of information known about p53 at the protein level. However, the molecular basis for transcriptional regulation of the *p53* gene has been less well defined. Therefore, understanding the transcriptional regulation of the *p53* gene can contribute to our understanding of the mechanisms involved in regulating the overall levels of p53 protein. It has been reported that, in many human cancers, *p53* transcription is deregulated (Boggs and Reisman, 2006, 2007; Khoo et al., 2009), whether it be increased (as in the case with mutant *p53* transcription) (Balint and Reisman, 1996) or decreased (failure to activate transcription of wild type *p53*) (Raman et al., 2000), and can contribute to tumorigenesis (Boggs and Reisman, 2006; Vousden and Lu, 2002).

The *p53* gene contains no obvious CAAT or TATAA motifs upstream of its transcription start site (Means and Farnham, 1990), but does contain a sequence that is homologous to an initiator element (Smale and Baltimore, 1989). Like many TATA-box-less promoters, the *p53* promoter contains sites of GC-rich content and a Sp1 binding site (Lee et al., 2001). The *p53* promoter becomes transcriptionally activated through the recognition of various transcription factor binding sites (Lee et al.,

Abbreviations: bp, base pairs; *E. coli*, *Escherichia coli*; EDTA, ethylenediaminetetraacetic acid; EMSA, electrophoretic mobility shift assay; FBS, fetal bovine serum; h, hour; HSV, herpes simplex virus; Nspc, non-specific competitor; Nuc, nucleotide; S phase, synthesis phase; Spc, specific competitor; SV40, simian virus 40; TBE, tris borate ethylenediaminetetraacetic acid; TK, thymidine kinase; TSS, transcription start site; WT, wild type; β-gal, β-galactosidase.

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2001). To date, multiple transcription factors have been shown to bind to the mouse *p53* promoter and participate in regulating the murine *p53* gene (Loging and Reisman, 1998). Some examples of these include USF, c-Myc/Max, ETF, NF-κB, NF1, YB1 (Lasham et al., 2003), PF2 (p53 factor 2) (Hale and Braithwaite, 1995), HoxA5, PBFI and PBFII [p53 binding factor I and II, respectively (Lee et al., 2001; Roy and Reisman, 1996)], and most recently C/EBPβ-2 and RBP-Jκ (Polson et al., 2010) (Loging and Reisman, 1998; Saldana-Meyer and Recillas-Targa, 2011) (Fig. 1A).

Recently, two genes located near the *p53* gene and positioned in the opposite orientation have been identified. The most well studied to date, termed *Wrap53*, partially overlaps the first exon of *p53* and encodes an antisense transcript that regulates *p53* post-transcriptionally (Mahmoudi et al., 2009, 2010; Polson et al., 2011; Venteicher and Artandi, 2009). The *Wrap53* gene undergoes complex alternative splicing, producing at least seventeen different splice variants (Mahmoudi et al., 2009). One or more of the splice variants of *Wrap53* encodes a WD40 domain protein that appears to be essential for Cajal body formation and telomerase activity (Mahmoudi et al., 2009, 2010).

The origin of the transcripts from this region appears to be complicated (Mahmoudi et al., 2009). The *Wrap53* gene has three alternative start exons: exon 1α, 1β, and 1γ (Mahmoudi et al., 2009). Exon 1α overlaps the first exon of *p53*, and exon 1γ overlaps the first intron of *p53* (Mahmoudi et al., 2009). Exon 1β of *Wrap 53*, however, is located downstream of exon 1α, and does not produce transcripts that are complementary to any p53 mRNAs (Mahmoudi et al., 2009). Since the protein coding sequences initiate in exon 2 of the *Wrap53* transcript, and are derived, in part, from transcripts that appear to initiate over 2000 bp downstream of the region of overlap with *p53* exon 1, the protein may, in fact, be encoded by a separate transcription unit that has been identified previously as *WDR79 (TCAB1)* (Alonso et al., 2009; Garcia-Closas et al., 2007; Mahmoudi et al., 2009; Venteicher and Artandi, 2009). Our identification of a transcriptional promoter that controls the expression of WDR79, separate from the control of the transcription of the *p53* regulatory *Wrap53* transcript, supports this conclusion (Polson et al., 2011).

The TSSs of the murine *p53* promoter and the *Wrap53/WDR79* promoter are separated by approximately 930 bp, in a head-to-head fashion (GenBank Database, 2010; Polson et al., 2011, http://www.ncbi.nlm.nih.gov/genbank/). Here, we focus our studies on the shared region between the TSS's of the mouse *p53* and the *WDR79* genes. It is possible that the *WDR79 (TCAB1)* gene may, in fact, map to the same location as *Wrap53β*, and that confusion in the literature has arisen because of the additional nomenclature. Therefore, what was previously reported by our group as the *p53/WDR79* bidirectional promoter will herein be referred to as the *p53/Wrap53β* bidirectional promoter (Polson et al., 2011). By focusing our studies on the transcriptional regulation of *p53* and its bidirectional gene partner, *Wrap53β*, we hope to elucidate key factors that can control and regulate the expression of these two genes.

Based on the results of mutational studies reported here, we were able to establish five different regions within the promoter that showed a loss of both promoter activities when the particular sequences were deleted from the *p53/Wrap53β* promoters. This loss of activity in these promoters led us to conclude that those deleted regions serve as a regulatory site within the *p53/Wrap53β* bidirectional promoter and play a role in regulating transcription of both the *p53* and *Wrap53β* genes. Further evaluation of these deleted sites led us to perform electrophoretic mobility shift assays (EMSA) to examine the binding of potential transcription factors to the *p53/Wrap53β* bidirectional promoter. Our results have demonstrated that two novel factors are binding to two different regions approximately 940 base pairs (bp) and 1185 bp upstream of the transcription start site of the *p53* gene.

2. Materials and methods

2.1. Bidirectional expression vector

The "pLucRLuc" bidirectional expression vector was created in the manner as reported previously (Polson et al., 2011). The mouse p53 promoter preparation was also reported previously (Polson et al., 2011).

2.2. Mouse p53 promoter restriction site deletions

The bidirectional murine *p53/Wrap53β* promoters cloned into the pLucRLuc vector were double digested with Bsu36I and Tth111I,

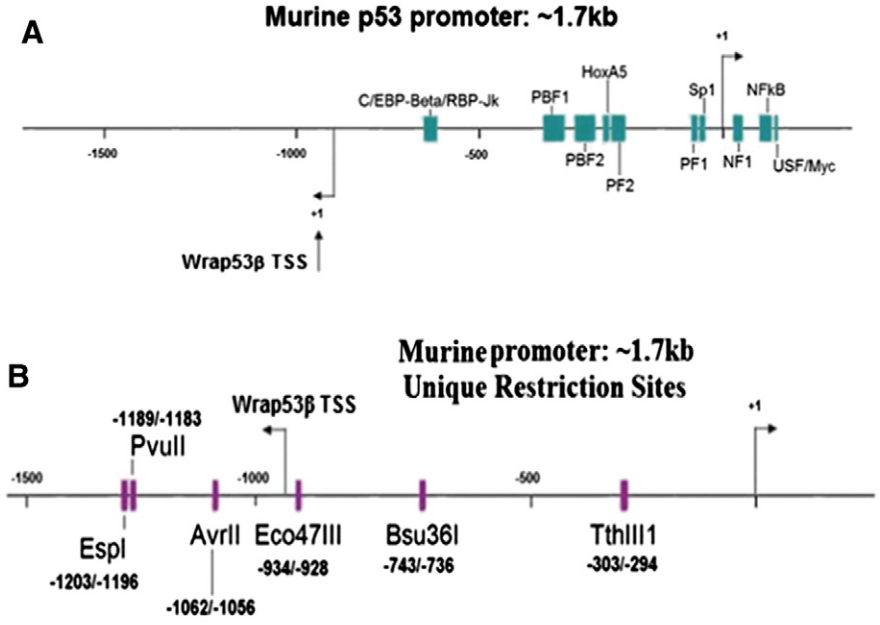

Fig. 1. *Known transcription factor binding sites on the p53 promoter that have been shown to regulate expression of the p53 gene, and the murine p53 promoter containing the unique, single cutting restriction sites.* (A) Schematic representation of the murine 1.7 kb *p53* promoter region and the location of known transcription factor binding sites. These factors have been shown previously to bind to the promoter region and regulate expression of the *p53* gene. (B) A schematic representation of the murine 1.7 kb promoter containing the unique, single cutting restriction sites present upstream of the *p53* TSS. The nucleotide positions where each restriction site is located on the promoter are shown and represent the positions that were deleted out of the *p53/Wrap53β* bidirectional promoter. The TSS for the *Wrap53β* gene is also shown.