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The Bi-directional Nature of the Promoter of the p53 Tumor Suppressor Gene

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p53 Promoter and the Transcriptional Regulation of the p53 gene

The expression of the p53 tumor suppressor gene is tightly monitored, and this serves as a mechanism to ensure genomic stability prior to cells entering S-phase, and to make ensure that the protein is rapidly induced in response to DNA damage [1]. In addition to alterations in protein stability, it is generally accepted that regulation of the p53 protein levels is also controlled at the transcriptional level [1,2].

The p53 gene contains no obvious CAAT or TATAA motif upstream of its transcription start site, but does contain a sequence that is homologous to an initiator element [3]. Like many TATA-box-less promoters, the p53 promoter contains sites of GC-rich content and a Sp1 binding site [4]. The p53 promoter becomes transcriptionally activated through the recognition of various transcription factor binding sites [4].

The well characterized murine and human p53 promoters are approximately 75% homologous and are off-set by about 110 base pairs (bp), relative to their transcription start sites (TSS) [5]. Due to this shift in sequences, some of the transcriptional regulatory sites in the mouse promoter, for example, c-Myc, map upstream of the TSS, but map downstream of the TSS in the human promoter [5,6]. With this off-set TSS of the two promoters, it is the general consensus that the functional promoter of the murine p53 gene extends approximately 100bp downstream of the TSS [5,6].

To date, multiple transcription factors have been shown to bind to the mouse p53 promoter and participate in regulating the murine p53 gene [5]. Relative to the TSS of the murine p53 gene, the USF and c-Myc/Max binding sites map at positions +75 to +70 and are necessary for maximal p53 promoter activity [5,6]. ETF, a GC-rich binding factor, maps at positions +66 to +63, while NF-κB (+68 to +49) transactivates the p53 promoter [5,6]. Other downstream binding factors, such as NF1 (+17 to +15) have also been demonstrated to bind to and regulate the murine p53 promoter [5,6].

There are also several factors that have been shown to bind upstream of the p53 gene, within the promoter region, and play a role in regulating the expression of the p53 gene [5,6]. YB1 binds to the murine p53 promoter at positions -127 to -136, and aids in the repression of transcription of the p53 gene [7]. The transcription factor PF2 (p53 factor 2) binds at positions -170 to -195 and has been shown to enhance expression of the p53 gene [8]. The homeobox protein, HoxA5, binds at positions -202 to -210, while two other factors, PBFI and PBFII (p53 binding factor I and II, respectively) bind upstream of the HoxA5 site, which appear to be highly conserved across multiple species [4,9] (Figure 1).

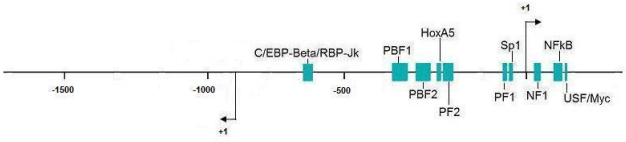


Figure 1: Relative positions of transcription factor binding sites on the murine p53 promoter. The arrow placed in between the Sp1 and NF1 sites represents the TSS of the p53 gene. The arrow that is upstream of C/EBPβ/RBP-Jκ binding site and in the opposite orientation of the p53 TSS corresponds to the TSS of the Wrap53/WDR79 gene as discussed below.

In addition, the transcription factor C/EBPβ-2 (CCAAT enhancer-binding protein β-2) binds to a regulatory site that is approximately 960 bp upstream of the transcription start site on the murine p53 promoter in response to mitogen stimulation in a cell cycle dependent manner [10]. This binding of C/EBPβ-2 to the p53 regulatory site positively regulates transcription of the p53 gene as cells are induced to enter S phase [10,11]. In numerous transformed cells that are defective in proper p53 regulation, this pattern is lost and aberrant expression of the p53 is seen as cells enter S-phase [10,11].

Finally, a negative regulator of p53 transcription, RBP-Jκ (CBF1), can also bind to the same regulatory site approximately 960bp upstream of the p53 transcription start site and repress p53 expression [12]. And it appears that a coordinated expression of these two transcriptional regulators (C/EBPβ-2 and RBP-Jκ) may be responsible for regulating p53 transcription as cells leave G0 and enter S-phas [13].

Bidirectional Activity of the p53 Promoter

Recently, two genes located near the p53 gene and arranged 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 [14,15]. In addition, the Wrap53 gene undergoes complex alternative splicing, producing at least seventeen different splice variants [14]. One or more of the splice variants of Wrap53 encodes a WD40 domain protein that appears to be essential for Cajal body formation [14,15].

The origin of the transcripts encoding the Wrap53 protein appears to be complex and some of the identified transcripts are likely to be initiated from a second promoter. 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 2000bp downstream of the region of overlap with p53 exon 1, the protein may be encoded by a separate transcription unit that has been identified previously as

WDR79 [14,16,17]. The promoter for Wrap53, therefore, is likely to map near the first exon of p53. Therefore, the TSSs of the human and murine p53 and WDR79 genes are separated by approximately 800bp and 930bp, respectively, in a head-to head fashion, and fit the criteria of what is designated to be a putative bidirectional regulatory region (Figure 2).

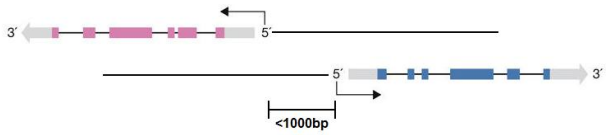


Figure 2: Representation of the head-to-head positioning of a pair of divergent genes and the bidirectional regulatory region separating them. The figure represents a hypothetical demonstration of how two oppositely oriented, divergent gene pairs may look. They are arranged in a head-to-head (5'-5') orientation, with the TSSs of the two genes separated by approximately 1000bp.

Previous computational analysis of many vertebrate genomes has identified a class of regulatory regions that are arranged in a head-to-head (5'to 5') orientation [18-20]. These regulatory regions often have fewer than 1000 base pairs (bp) separating their corresponding transcription start sites and have been termed as being "bidirectional" [18,20-22]. This bidirectional arrangement and the divergent gene pairs (bidirectional genes) under the control of these regulatory regions (bidirectional promoters), are found, not only the human genome, but also in other genomes, such as the mouse, yeast, and plants [18,23].

Since divergent transcription occurs more often than was previously thought, defining features of these divergent or bidirectional promoters is crucial in functionally understanding how these particular elements operate. Shared bidirectional regulatory regions, such as gene promoters, can influence the expression of the oppositely oriented genes, yet many of the specifics as to how this process occurs or is regulated is unknown [24].

In order to determine whether th ep53 promoter functions in a bi-direc tional fashion, we constructed a bidirectional reporter vector, termed pLucRLuc, To generate the vector, shown in Figure 3, the Renilla reporter gene [25] was combined with the pGL3 luciferase reporter vector (Promega) to generate a novel vector expressing the two reporter genes in opposite orientations [13] (Figure 3).

The p53/Wrap53(WDR79) regulatory element is bidirectional

Mouse and human p53/WDR79 promoters were cloned into the pLucRLuc bidirectional expression vector at the Kpn1 cloning site. The DNA containing the mouse or human bidirectional p53/WDR79 promoters and a control β-Gal expressing construct were transiently transfected into murine Swiss 3T3 fibroblasts and human U2OS osteosarcoma cell lines, respectively. Extracts were obtained and Luciferase, Renilla, and β-Gal activity was assayed and the activity of the two bioluminescent reporters was measured in order to determine the transcriptional output from each of the promoters. As seen in Figure 4, both the murine and human Wrap53/WDR79 and p53 promoters show an increase in transcriptional activity in a dose dependent manner.

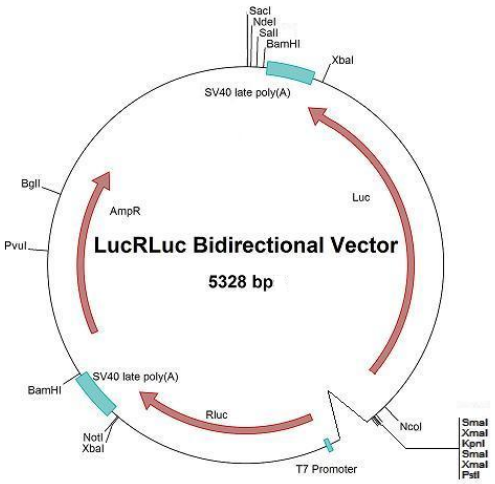


Figure 3: pLucRLuc bidirectional reporter vector.

This is consistent with the previous findings assaying the individual promoter activities using the pGL3 expression vector (data not shown). These results demonstate that the promoter sequences upstream of both the human and mouse p53 genes function bidirectionally and that the pLucRLuc bidirectional expression vector is a functional vector capable of assaying the transcription from bidirectional promoters. In addition, based on an extensive series of additional experiments, it was further determined that the bidirectional promoter of the p53 and Wrap53/WDR79 genes is regulated in a similar manner in numerous cell types, as cells enter S-phase and by many of the same transcription factors [26].

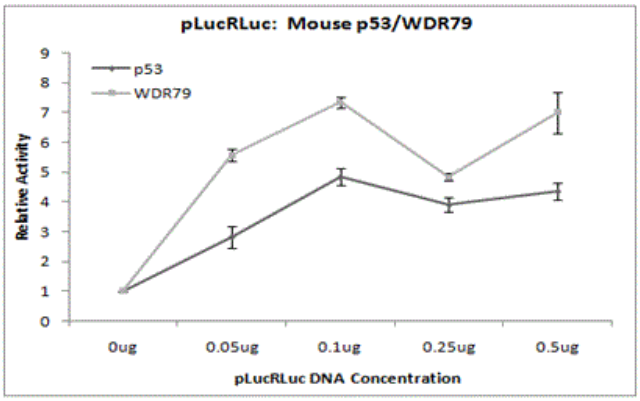
Conclusions

The maintenance and expression of the p53 tumor suppressor is critical to the prevention of cancer. A wealth of information is known about the p53 molecule at the protein level and how it is modified to exert its mechanisms of actions; however, there is incomplete information in the literature about how this important tumor suppressor is transcriptionally regulated. By focusing our studies on the transcriptional regulation of the p53 gene, we have been able to identify various factors that are involved with its transcriptional regulation.

Also, analysis of many vertebrate genomes has identified a class of regulatory regions that contain a head-to-head (5' to 5') arrangement on opposite strands of the DNA [18-20]. Discussed in this review is the identification of one such bidirectional regulatory promoter that is responsible for regulating the p53 and Wrap53/WDR79 genes.

The Wrap53/WDR79 transcript encodes a WD40 domain containing protein that can have a variety of functions ranging from signal transduction and transcriptional regulation to cell cycle control [14,27]. In particular it appears to be essential for Cajal body formation [14,15] and to be a component of active telomerase and to be required for the telomerase holoenzyme (TERC, TERT, and

dyskerin) that accumulates in the Cajal bodies and elongates telomeres [27]. Elongation of telomeres by telomerase is a characteristic that is observed in many cancers, whereas normal cells progressively lose telomeres with each successive cellular division until they reach a growth arrest [28].



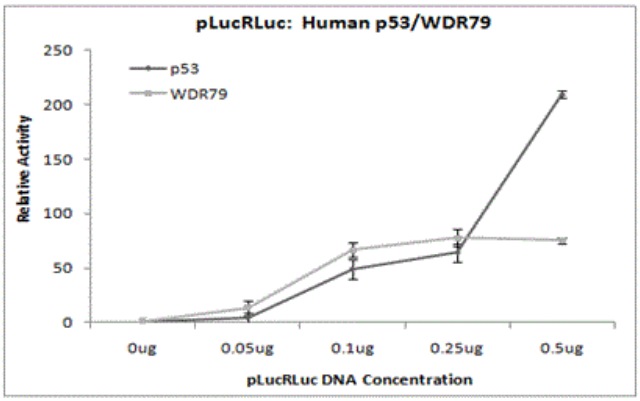


Figure 4: pLucRLuc DNA concentration.

What these findings may indicate is that transcripts derived from promoters in close proximity to the p53 tumor suppressor gene may also be important in the maintenance of cancer cells, possibly allowing cells to take on an oncogenic function [29]. These predictions are consistent with previous findings stating that proteins derived from transcripts within this region of the genome are upregulated in many human cancers [29].

It has been previously determined that there an upregulation of p53 in many human cancers, but this upregulation is typically of the mutant form [30]. Therefore, it is possible that the combination of expression of mutant p53 and the upregulation of Wrap53/WDR79through the action of the bi-directional promoter allow cells to evade anti-growth and anti-apoptosis signals.

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