DISCUSSION
5.0 DISCUSSION

Gene expression is controlled at several steps. Transcription is one of the important steps at which regulation occurs. DNA-binding proteins are important for regulating gene expression during development. It is widely assumed that this regulation involves sequence-specific DNA binding by the transcription factors to cognate cis-regulatory sequences of their downstream target genes (Segal, 2003).

Transcriptional activation of eukaryotic genes during development or in response to extracellular signals involves the regulated assembly of multiprotein complexes on promoters. The complex nature of this process are sequence specific transcription activators that select genes to be activated. These activators and coactivators, including TAFs, SRB complex/mediator orchestrate the assembly of a transcription machine at the start site of mRNA synthesis or mediate the binding of a preassembled complex to its template DNA. Negative cofactors including repressors can block formation of the preinitiation complex at multiple steps (Kovacs, 2003).

A key issue in understanding this process is how a relatively small number of different activators and repressors can be used to achieve the high level of specificity required to regulate the complex patterns of gene expression in higher eukaryotes. At least part of the answer is that activators and promoters (and also repressors) are composed of modular components. For example, a typical activator contains a specific DNA-binding domain that directly contacts DNA, a multimerization (dimerization) domain that allows the formation of homo- or heteromultimers, and a transcriptional activation domain (Segal, 2003). Importantly, these domains can be combined in a modular fashion to generate novel and fully functional transcription factors. Similarly, promoter contains distinct sets of activator binding sites and repressor binding sites (silencers).

Variations in the arrangement of the binding sites provide the potential to create unique nucleoprotein complexes by forming heterodimers within and among activators and repressors. Synergistic interactions between the proteins within the complex result in (i) specificity, (ii) a potential for multiple regulatory controls, and (iii) a high level of transcription.

The regulatory logic and underlying mechanisms of eukaryotic gene regulation are ideally suited for achieving extremely complex patterns of gene expression. Tumor suppressors such as p53, which is basically a transcription
factor, perform its function primarily by the transcriptional activation of the target genes. p53-tumor suppressor has been identified as a key player in our body's built -in scheme of cancer prevention. Consequently, direct mutational inactivation of the p53 gene was found to be the most frequent single genetic alteration associated with human cancer, occurring in about half of all individual tumors (Zhao, 2002). The great interest in p53, spurred by the realization of its pivotal relevance to human cancer, has generated a food of information addressing almost any possible aspect of p53 biochemistry and biology. Among the main findings was the fact that the p53 protein is by and large a sequence-specific transcription factor that is capable of activating the transcription of adjacent genes, upon binding to defined consensus sites within the DNA (Donehower, 1993).

Several oncogene products function as transcription factors and have been shown to auto regulate their own transcription. Examples include the products of the c-myb and c-jun oncogenes which up regulate their own promoters, whereas, the product of the c-myc and c-fos oncogenes down regulate their own transcription. Till date studies concerning the auto regulation of p53 gene do not give consent on whether the product of p53 gene transactivates or down regulate its own transcription. The promoter region of a gene plays a major role in its transcriptional regulation, so it would be very useful to study the presence of p53 DBS in the promoter region of p53 gene.

Our result demonstrates that p53, by binding to p53 DBS in its upstream regulatory region (fig.10), does auto-regulate its own transcription. p53 gene promoter (fig.14), P1, contains three p53 DNA-binding sites that share 80% homology with the p53 DBS consensus sequence Pu Pu C (A/T) (T/A) G Py Py Py (Reisman, 1998). It has been proposed that there are two classes of p53 DBS that provide a mechanism for target gene selectivity (Resnick-Silverman, 1998). The present study shows that p53 DBS - I and III, that does not contain a 'C' at the fourth position of the pentanucleotide Pu Pu Pu C (A/T), belong to class II of p53 DBS whereas p53 DBS II falls under class I of p53 DBS (fig.8a). The classification of Class I and II has been based upon a fact that the binding of recombinant p53 wt to p53 DBS I and III (fig. 8b) was inhibited by addition of a monoclonal antibody mAb421 that was raised against the C-terminal domain of p53. On the contrary, the binding to p53 wt to DBS II was enhanced in the presence of mAb421 through an unknown mechanism. The DBS III with the flanking
region from -384 to -417, that we have discovered, activates p53 gene promoter in
various cell lines and the transactivation was dependent on the endogenous wild
type p53 level. The earlier transfection studies on p53 DBS I and II in various cell
lines had demonstrated that p53 DBS I significantly activated p53 gene promoter
whereas p53 DBS II both activated and repressed p53 gene in a cell-type specific
manner (Deffie, 1993; Benoit, 2000). Our results now demonstrates that p53 in
association with an 80 kDa protein activates p53 gene (fig.15). p80 binds to
further upstream of DBS III (fig. 13) and associates with the C-terminal domain of
p53 (fig.18). Further, p53 in association with HDAC1 represses its own
transcription.

The present study shows that the p53 binds to DBS I, II and III of the p53
gene. Invariability of the conserved bases within p53 DBS is crucial for the
specific interaction with target sites whereas the non-conserved bases can be
rather variable (Qian, 2002). It was proposed earlier that the sequence variance of
DBS might be an important parameter in determining p53 interactions with DNA
as it provides DNA with structural flexibility (Resnick-Silverman, 1998). The
nucleotides G and A residues at the position 2 and 5 of the consensus pentamer
sequence are critical determinants of the p53 DNA binding and changing of A
residue at position 1 enhances the binding (Lee, 1995). Further, it was reported, in
an yeast transactivation assay, that approximately 55 % of p53 DBS has a G
residue at the positions 1, 2 and 3, 98 % has C at position 4, and 77 % have got A
residue at position 5 of the pentamer (Gohler, 2002).

There is growing evidence that the p53 tumor suppressor protein not only
can function to activate gene transcription but also to repress the expression of are
specific genes (Ko, 1996; Murphy, 1998). Histone deacetylases are active
components of transcriptional corepressor complexes. Mammalian HDAC1, -2,
and -3 are known to specifically downregulate the transactivation activity of p53
(Juan, 2000). Most importantly, the downregulation of p53 function by HDACs
relies largely on the C-terminal 30 residues of p53, the region containing the basic
lysines (Lys-373 and Lys-382) that are acetylated by p300/CBP in vivo
(Bannister, 1996; Ogryzko, 1996; Liu, 2003). In the present study, we have also
demonstrated that HDAC1 represses p53 gene and thereby form a p53 auto-
regulatory loop (fig. 19; fig.20). Further, the deletion of C-terminal domain
elevates this repression (fig.16). Based on the above study a model could be
hypothesized for p53 protein in which p53 and p80 proteins bind to the upstream regulatory region of p53 tumor suppressor gene and up-regulate its transcription (fig. 23). Any external change or mitogenic stimulation might allow p53 to interact with p80 in order to up-regulate itself and thereby increasing and decreasing its level in response to cellular

![Autoregulatory model for p53 gene expression](image)

Fig. 23 Autoregulatory model for p53 gene expression. p53 and p80 proteins bind to the upstream regulatory region of p53 gene to bring about its transcriptional upregulation. p53 Δ287 is unable to interact with p80 due to loss of regulatory domain leading to increase level of p53. p53wt interacts with HDAC1 and bind to p53 DBS thereby repressing its own synthesis.

requirement. p53Δ287-393 is unable to interact with p80 and hence a loss of regulatory domain leads to an increase in the p53 level. HDACs may also play a role here by repressing the transactivation function of p53 protein. Wild-type p53 interacts with HDAC1 to repress its own synthesis.

Wild type p53 can modulate the transcription of a vast number of target genes that have roles in diverse cellular processes, including cell cycle control, apoptosis, senescence, differentiation, DNA repair and in its own turnover and activity. The differential trans-activation by p53, in terms of both selectivity and kinetics of gene activation and repression, is important in determining which of these various processes is elicited, particularly, with respect to the choice between cell cycle arrest and apoptosis. The presence of two classes of p53 DBS in the p53 promoter suggests a possible mechanism of wild type p53 to modulate its own

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transcription to affect the turnover of p53 protein. DBS II is located 50 bp 5’- to the DBS I and 153 bp 3’- to the DBS III. The close proximity of the three sites suggests that p53 might choose any of these sites depending on its conformational state. As DBS I is close to the transcription start site, we propose that p53 alone in a specific conformation state might choose this site for transcriptional activation. However, in choosing the sites DBS II and III it might require an additional coactivator or corepressor. In our experiments, we have shown that p53 either choose p80 as a coactivator or HDAC1 as a corepressor in contacting DBS III. It was previously reported that NFKB was recruited as a coactivator with DBS II (Benoit, 2000). p53 promoter contains one DBS of class I type and two DBS that belong to class II. We propose that an auto-regulatory model might exist for p53 transcription in which p53 might interact with both types of DBS in order to regulate its own transcription in response to stress. Our finding that an additional p53 DBS is involved in the activation and repression of p53 gene is interesting. The role of the DBS III in association with the two other DBS may throw some light upon the regulation of cell cycle and cell death via p53.