

# [Unique function of p53 homolog p63](https://assignbuster.com/unique-function-of-p53-homolog-p63/)

P53 homolog p63has a unique function

P53 is commonly known as a transcription factor and is well characterized for its role in DNA damage and cell cycle control (1), but less is known about its related homologous genes, p63 and p73 (2).  While p63 and p73 are very similar in structure to p53, recent literature suggests that these homologous forms may have a unique function (3).  The homologous genes share similar domains, which include a transactivation domain, a proline-rich domain, a DNA-binding domain, a tetramerization domain and lastly a carboxy-terminal regulatory domain (4).  P63 has several different isoforms that have been identified with commonalities in the DNA-binding domain (3).  The homolog p63 contains the same conserved residues for zinc binding as the p53 protein, indicating that p63 may bind zinc and have DNA binding functions similar to p53 (5).  P53 and p63 do share similar functions, such as binding to various downstream targets, including PG, Bax, Puma, and p21 (3, 6) but p63 also appears to have independent functions.  P63 has been implicated iin early development (7).  Upon disruption of the p63 gene, prominent defects in embryonic mice were observed (7).

This work set out to identify if the DNA binding domain of p63 is similar in function to the DNA-binding domain of p53 and to determine if the homologous p63 can bind to DNA following a similar mechanism as p53.  The constructs of the p63 DNA-binding domain used were based on the alignment similarity to the DNA binding domain of p53, and the specific isoform of p63 studied was the p63 gene from the human placenta sample, classified as p63- δ (2).  Both DNA binding domains of p63 and p53 were approximately over 50% identical (2).

To test the binding affinity of p63 to DNA, an electromobility shift assay (EMSA) was performed.  The authors tested the ability of p53 and p63 DNA-binding domain constructs to bind to PG (polygrip) p53 consensus oligonucleotide sequence (2, 8).  For this experiment, 50 ng of protein was incubated 10 ng of PG oligonucleotide and 5 nM of non-specific competitor DNA as a control. The p53 DNA-binding domain was able to bind to the consensus sequence with high affinity, however the p63 DNA-binding domain failed to bind the consensus sequence.  The authors then tested p63 and p53 constructs that were linked to a GST tag (2).  The advantage of these constructs was that the GST tag allowed for self-dimerization, and because p53 and p63 are believed to bind DNA in a tetramer form (2), this should improve binding to DNA.  When the GST- constructs were tested, both p63 and p53 bound to the consensus sequence with comparable affinities.  Based on these results, the authors were concerned that the GST tag may change the conformation of p53 and p63.  In order to test if the conformation was altered, GST was removed using thrombin (which cleaves following an Ile, Glu, Gly, Arg site in the linker (9) between the GST tag and the p53/p63 construct).  The p53 with cleaved GST still bound to the consensus sequence with the same affinity as the control p53 construct, indicating that the GST tag was not causing a conformational change of the DNA binding domains to impact function.

As mentioned previously, zinc is necessary for normal p53 binding to DNA.  The authors hypothesized that zinc may also be a requirement of p63 for binding to DNA (2).  The authors tested the effect of 1, 10-phenanthroline, which is a metal-chelator, in altering DNA binding activity of GST-p53 and GST-p63.  With high amounts of 1, 10-phenanthroline, the p53 constructs bound the consensus sequences with less affinity (2).  The GST-p63 construct responded in a similar manner.  Very low amounts of 1, 10-phenanthroline was able to abrogate GST-p63 binding to DNA, while the p53 constructs required higher amounts of 1, 10-phenanthroline, indicating that p53 binds zinc more tightly than p63 (2).

Based on these results, it can be concluded that p63 may have a unique function, as it does not bind the p53 PG consensus sequence with high affinity unless a GST tag forcing dimerization is present.  In addition, the authors also found that the p63 DNA binding domain construct had enhanced thermostability (2).  These results indicate that p63 may bind DNA with a lower affinity than p53, or p63 may have an altogether different function (2).   Further work has proposed that p63 may have a p53 independent preference in binding of downstream targets and may have a more dominant role in development, rather than cell cycle control (3).  P63 is rarely mutated in cancers, unlike p53, indicating that it may have only a minor function in tumorigenesis, however further work needs to be done to fully elucidate this role (10).

## References

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