

HUMAN Δ Np73 REGULATES A DOMINANT NEGATIVE FEEDBACK LOOP FOR Tap73 AND p53

Grob, T.J. *et al.* (2001) *Cell Death Differ.* 8, 1213–23.
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This article highlights the use of a variety of Promega molecular biology products to answer questions about the regulation of apoptosis by p73 and its splice variants.

One of the most common genetic events in the development of cancer is the mutation of the tumor suppressor p53. In normal cells, p53 gene expression is induced in response to several forms of genotypic stress and subsequently results in apoptosis or cell cycle arrest. Therefore, p53 expression has to be regulated to avoid the uncontrolled induction of apoptosis. The pathways that regulate p53 expression are complex and can involve important feedback loops.

p73 Regulates Apoptosis

Researchers are investigating the homology between sequences of p53 and a second protein p73. Although the domain organization of both of these proteins is similar, transgenic studies indicate that mice knockouts of the two genes have distinct phenotypes. Other studies have demonstrated that p73 is involved in a rescue pathway following DNA damage that, similar to the p53 pathway, induces apoptosis.

Mice and humans express N-terminal splice variants of the p73 protein. Two of these splice variants lack the trans-activation domain and consequently inhibit the activity of the full-length p73 protein and p53, acting as dominant negatives. Moreover, these variants can inhibit p53-mediated apoptosis. In functional studies of p73, the authors reproduced the dominant negative role of the p73 variants using cotransfection experiments in which a construct, carrying the luciferase reporter under the control of the p21 promoter, was cotransfected with vectors expressing p53 and the p73 variants. These reporter experiments conclusively demonstrated the capacity of p73 variants to inhibit p53-induced expression.

Expression of the p73 variants appears to be regulated at the transcriptional level, and this regulation involves a second promoter, P2. These authors determined that the human exon 3 sequence contains two in-frame ATG start codons from which translation can begin. They confirmed translation from these two codons by Western analysis of protein translated *in vitro* using the TNT[®] Quick Coupled Transcription/Translation System (Cat.# L1170, L2080).

In their studies, the authors demonstrated the transcriptional regulation of p73 variants by p53 and p73

and they demonstrated the binding of these proteins to the P2 promoter. They also described a classical feedback loop in which expression of p53 and full-length p73 are similarly inhibited at the transcriptional level by the p73 variants. Given this inhibitory activity, the p73 variants may play an important role in the onset of cancer.

The authors investigated the relative levels of both the full-length and variant p73 mRNA in a variety of cells and tissues. Using real-time RT-PCR^① they showed that the level of p73 mRNA is much higher than the level of variant p73 mRNA in most tissue types. Moreover, the expression of both of these mRNAs was considerably higher in fetal tissue than in adult tissue, indicating the possible involvement of variant p73 in fetal development. The RiboMAX[™] Large Scale RNA Production System (Cat.# P1280, P1300) was used to synthesize competitor RNAs in this quantitative RT-PCR procedure.

Reporter experiments revealed a putative 150bp sequence in the P2 promoter through which p73 and p53 proteins regulate p73 variant expression. For these reporter assays, the pGL3-Basic Vector (Cat.# E1751) was used for cloning the promoter sequences. The firefly luciferase activity was normalized against *Renilla* luciferase using the Dual-Luciferase[®] Reporter Assay System (Cat.# E1910).

Ordering Information

Product	Size	Cat.#
TnT [®] T7 Quick Coupled Transcription/Translation System ^{*(a,b,c,d,e)}	40 reactions	L1170
TnT [®] SP6 Quick Coupled Transcription/Translation System ^{*(a,b,c,d,e)}	40 reactions	L2080
RiboMAX [™] Large Scale RNA Production System-SP6 ^{*(c,d,e)}	1 system	P1280
RiboMAX [™] Large Scale RNA Production System-T7 ^{*(c,d,e,f)}	1 system	P1300
pGL3-Basic Vector ^(d,g)	20µg	E1751
Dual-Luciferase [®] Reporter Assay System ^(a,h,i)	100 assays	E1910

*For Laboratory Use.

① U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289 and 5,814,471, Australian Pat. No. 649289 and other patents and patents pending.

② U.S. Pat. Nos. 5,324,637, 5,492,817, and 5,665,563, Australian Pat. No. 660329 and other patents.

③ U.S. Pat. No. 5,552,302, Australian Pat. No. 646803 and other patents.

④ The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

⑤ U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

⑥ The RiboMAX[™] Large Scale RNA Production System—T7 (Cat.# P1300) is covered by U.S. Pat. No. 5,256,555 and is sold under a license from Ambion, Inc. It is intended for research use only. Parties wishing to use this product for other applications should contact Ambion, Inc.

⑦ U.S. Pat. No. 5,670,356.

⑧ Certain applications of this product may require licenses from others.

⑨ U.S. Pat. No. 5,744,320, Australian Pat. No. 721172 and other patents pending.

⑩ The PCR process is covered by patents issued or applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.