F111 Cell Cycle-Dependent Regulation of the Rat DNA Topoisomerase II α Gene Promoter

Jeong Ho Yoon*[1], Mi Sook Oh [1], Seung Hwan Hong[1,2]
and Sang Dai Park [1]
Dept. of Molecular Biology and RCCD[1], IMBG[2], Seoul National University

DNA topoisomerase II α is a homodimeric nuclear enzyme required for many aspects of DNA transaction and its expression is tightly related to the growth state of and cell cycle of given cells. To verify the mechanism of transcriptional regulation of this gene, we have isolated 2.7 kb 5'-upstream genomic DNA fragment and determined its nucleotide sequence. It contains 4 copies of inverted CCAAT box (ICB), 2 copies of GC box (also termed Sp1), 1 copy of ATF and C/EBP within the 630 bp upstream from the first ATG codon.

By using transient expression system, we have evaluated the effects of these putative cis-acting elements on the transcription of the topo II α. The 5'- and 3'-serial deletion, and site-directed mutation experiments revealed that both ICB4 (-166 to -162 bp) and GC2 (-149 to -143 bp) are important for the cell cycle-dependent regulation of topo II α expression. [Supported by grants from KOSEF]

F112 Promotion of ceramide-induced apoptosis by p21 in human hepatocarcinoma cells

KANG Kyung-hwa, KIM Won-ho and CHOI Kyung-hee *Dept. of life science, college of natural sciences, Chung-Ang univ. Seoul, 156-756*

p21, a potent cyclin-dependent kinase inhibitor, has been known to induce cell cycle arrest in response to DNA damaging agent. Although p21 has been reported to play an important role in the regulation of apoptosis in some cells, postulated role for p21 in apoptosis is still controversial. Previously, we reported that p21 was induced by p53-independent manner during ceramide-caused apoptosis in human hepatocarcinoma cell lines. In the present study, we investigated the precise role of p21 in ceramide-induced apoptosis by using tetracycline inducible expression system. Overexpression of p21 by itself did not induce apoptosis in Hep3B cells, which do not express endogenous p21. However, Hep3B/p21 cells were more sensitive to ceramide-induced apoptosis. To study the molecular mechanism of apoptosis mediated by overexpression of p21 gene, the expression levels of Bcl-2, antiapoptotic protein, and Bax, proapoptotic protein, were measured. Expression level of Bax, but not Bcl-2, was significantly increased in Hep3B/p21 cells compared to Hep3B cells. On the other hand, Bcl-2 not only suppressed apoptosis but also completely prevented p21 induction caused by ceramide in SK-Hep-1 cells, another hepatocarcinoma cells. Furthermore, overexpression of p21 antagonized death protective function of Bcl-2 and up-regulated expression of Bax protein. Taken together, these results suggest that p21 promotes the ceramide-induced apoptosis by enhancing the expression of Bax, thereby modulating the molecular ratio of Bcl-2:Bax in human hepatocarcinoma cells.