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INHIBITORS OF DNA TOPOISOMERASE II INDUCE TOPOLOGICAL CHANGES IN AN ARCHAEBACTERIAL PLASMID IN VIVO

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The living cellular organisms are now divided into three distinct urkingdoms : the eukaryotes, the eubacteria and the archaebacteria (1). Although the archaebacteria are prokaryotes in terms of cellular organization, many features of their molecular biology resemble those of the eukaryotes.

It is becoming increasingly evident that DNA structure plays an important role in the control of gene expression, both in eukaryotes and prokaryotes.

In order to study DNA topology in archaebacteria, we have investigated the effect of topoisomerase II inhibitors on halophilic archaebacteria. Previous studies have shown that these organisms are sensitive both to inhibitors of eubacterial DNA gyrase and to inhibitors of eukaryotic type II DNA topoisomerases (2).

Interestingly, as in eukaryotes, the antitumoral drugs epipodophyllotoxins VP16 and VM26 stimulate DNA single and double stranded breaks and protein-DNA covalent linkage in the chromosome of Halobacterium halobium (3) and in the negatively supercoiled 1.7 kb plasmid of Halobacterium GRB. These breaks are not randomly generated as only three major cleavage sites can be detected on the 1.7 kb plasmid DNA after VP16 treatment. The cleaved plasmids contain protein linked to their 5' ends. In addition, to DNA breaks, VP16 treatment gives rise to positive and negative topoisomers which forme the right and the left branch of an arch respectively (fig. 1, panel A) following 2-D gel electrophoresis.

The DNA breaks are completely abolished by novobiocin, a drug which belongs to a different class of topoisomerase II inhibitors. In contrast to VP16, novobiocin interferes with the DNA topoisomerase II activity by affecting the ATPase function. The above results taken together suggest that, as in eukaryotes, the target of epipodophyllotoxins is a type II DNA topoisomerase.

In contrast to limited effect of VP16 on the 1.7 kb plasmid topoprofile, novobiocin induces positive supercoiling of all detectable molecules of the 1.7 kb plasmid or pHV1 plasmid DNA, a 6.3 kb plasmid from Halobacterium volcanii. Removal of novobiocin restores the original negative supercoiling of these plasmids.

These results suggest that the level of supercoiling in archaebacteria depends on the opposing activities of at least two topoisomerases : the first, a novobiocin sensitive enzyme, keeps the plasmid DNA in a negatively supercoiled state (type II DNA topoisomerase) while the second increases the DNA linking number (type I DNA topoisomerase).

The discovery of mechanism which creates positive superturns in halophilic archaebacteria fits well with the existence of a reverse gyrase in the archaebacterium Sulfolobus acidocaldarius (4,5) and positive supercoiling of the DNA from the archebacterial virus-like particle SSV1 (6). Since reverse gyrase is a type I DNA topoisomerase (5), it is tempting to suggest that inhibitors of an halobacterial type II DNA topoisomerase by novobiocin unmasks the activity of an halobacterial type I DNA topoisomerase with reverse gyrase activity.

In addition to positively supercoiled DNA, novobiocin treatment of Halobacterium GRB produces single stranded circular plasmid DNA which migrates faster through an agarose gel than supecoiled double strand DNA (fig. 1, panel B). This SS-1.7 kb plasmid DNA corresponds to only one of the two DNA strands. The induction of this SS-1.7 kb plasmid was abolished when the cells were treated prior to novobiocin treatment with either -aphidicolin or anisomycin, two drugs which completely inhibit halobacterial DNA replication and protein synthesis respectively (7,8). These results suggest that the SS-1.7 kb plasmid DNA is a

replicative intermediate and that DNA topoisomerase II is essential for the replication of the 1.7 kb plasmid. This intermediate normally has a very short half life in untreated cells.

Several lines of evidence indicate that topoisomerases affect transcription and replication through their effects on DNA superhelicity in both eukaryotes and prokaryotes. However, as yet, nothing is known about the role of archaebacterial DNA topoisomerases in gene expression. The 1.7 kb plasmid DNA appears to be an appropriate model for the investigation of possible roles of archaebacterial DNA topoisomerases in gene expression.

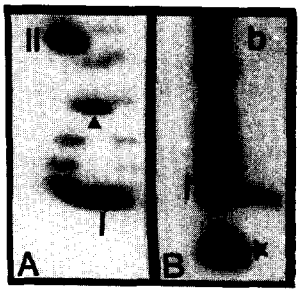

Figure : Analysis of the 1.7 kb plasmid from novobiocin and VP16-treated cells.
Panel A : plasmid from VP16-treated cells in gel containing 10 ng/ml of ethidium bromide in the second dimension which was run from the left to the right.
Panel B, lane a : plasmids from novobiocin treated cells, lane b : plasmids from untreated cells.

Methods : Novobiocin (1,5 ug/ml) or VP16 (100 ug/ml) were added to 5 ml cultures at an optical density of 0.2 (600 nm). After 16 hours, the cells were collected by centrifugation and lysed by addition of T.E buffer and 1 % SDS. The lysate was centrifuged in an eppendorf centrifuge to remove cell debris. Samples of the supernatant were loaded directly on a 1.2 % agarose gel. Electrophoresis, transfer of the DNA to nitrocellulose and hybridization with 1.7 kb plasmid DNA probe were performed as usual. The arrow indicates the linear form of the 1.7 kb plasmid DNA induced by VP16 treatment. The migration of highly negatively and positively supercoiled circular, and nicked circular plasmid DNA are indicated by I, I' and II respectively. The SS-1,7 kb plasmid DNA is indicated by *.

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