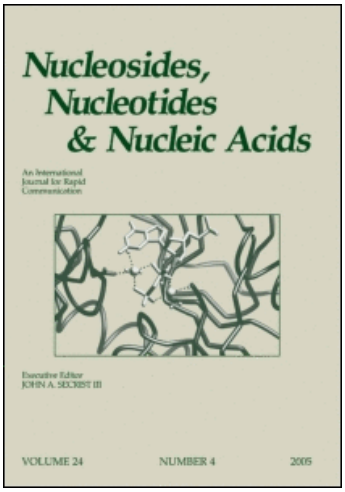

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Inhibition of DNA Transcription Elongation and Unwinding by Bis-Pna

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INHIBITION OF DNA TRANSCRIPTION ELONGATION AND UNWINDING BY BIS-PNA

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ABSTRACT.
The possibility to arrest transcription elongation by bis-PNA has been investigated. No arrest of transcription has been obtained in intact cells at variance with the data observed with purified RNA-polymerases. In keeping with these data a purified DNA-helicase (UL9) cannot be fully inhibited by such complexes.

Peptide nucleic acids (PNA) lead to sequence specific triple helix formation and strand displacement thus allowing interesting prospects for gene transcription inhibition[1, 2]. Interference with the assembly of a transcription initiation complex has been documented[3]. We have investigated a possible arrest of transcription elongation by bis-PNA which bind tightly to their DNA target (Tm of 86°C in our model system). A specific blockade of purified T3 and T7 RNA-polymerases has been obtained in agreement with previous data[4]. Preformed complexes between the targeted plasmid and bis-PNA have been transfected in various cell lines and monitored for ß-galactosidase at several time points. No inhibition of transcription activity has been observed at variance with cell free assays or with data obtained previously in the laboratory with psoralen-conjugated oligonucleotides[5].

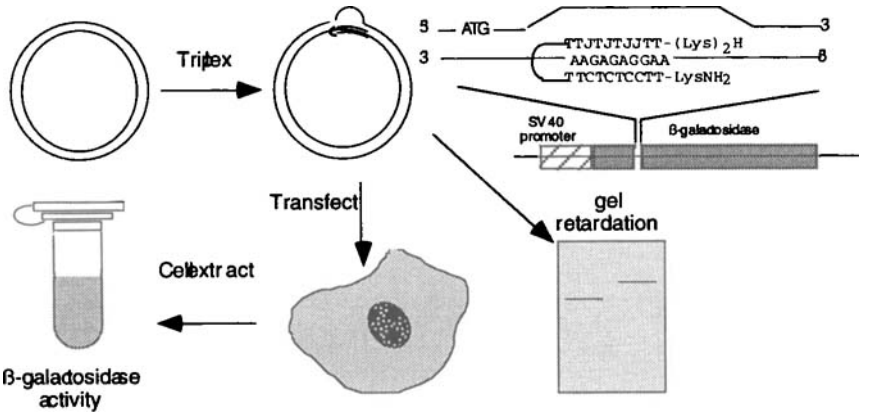

Figure 1. Schematic representation of the reporter plasmid and experimental strategy for probing triplex formation and transcription efficiency. Plasmid was obtained as previously described[5]. Triplex formation was initiated by addition of PNA (100 fold molar exces) to the plasmid. Gel retardation assay was used to determine the extent of triplex formation. Plasmid was transfected in CCL39 cells and Hela cells. After a period of time (24 , 48 or 72h), cell extracts were made for ß-galactosidase assay to measure transcription efficiency.

An extrapolation of the data obtained from cell-free assays to intact cells is often difficult since cellular metabolic processes such as transcription are mediated by many interacting polypeptides. These complexes deal more efficiently with secondary structures than isolated polymerases. Little is known in particular concerning the role of DNA-helicases in the unwinding of triple helices[6, 7]. In this respect, we have shown that the very stable complex formed between a bis-PNA and a DNA target cannot completely block the DNA-helicase activity of the well characterized herpes simplex virus type I UL9 protein[8].

Altogether these experiments point towards the difficulty of achieving a steric block of transcription elongation even when using very stable bis-PNA-complexes.

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