Decrease in gyrase A protein expression in *E.coli* cells inhibited by antisense ribozymes

Antisense molecules with guide sequences have several unique features as gene targeting agents. RNA based External Guide Sequences (EGS) have been expressed endogenously as transgenes in both bacteria and mammalian cells (1, 2). EGS has been effective in inhibiting the gene expression of herpes simplex and in inhibiting the replication of influenza virus in human cells (3, 4).

EGS based approach have also been used to inactivate essential gene products (5). Previous data from our laboratory had suggested downregulation of gyrase A gene expression in BL21 (DE3) cells transformed with the plasmid pT7EGSEGyrA (functional EGS against gyrase A) after 18 h of IPTG induction (6).

Because earlier work had suggested downregulation of gyrase A gene expression, we were interested to see the effect of protein expression of DNA gyrase that was inhibited by the antisense ribozymes. Growth conditions of the cell BL21 (DE3) were as described (5). EGS expression vector plasmid was received as a gift from Dr. Jeffrey McKinney (5). Overnight cultures of BL21 (DE3) containing plasmid vector encoding the inducible expression of various EGS oligonucleotides were each grown in LB carbenicillin liquid culture (LB medium supplemented with 50µg/ml carbenicillin). On entry into log phase liquid cultures received a fresh aliquot of carbenicillin stock (for an additional 5 µg /ml final concentration) and were split into two culture tubes. These split cultures were incubated in a 37 ºC shaker in parallel: one tube with the inducer, IPTG for 2mM final concentration for EGS production and one tube without. Cells (1x 10s cells/ml) were lysed in lysis buffer containing 20mM Tris-HCl pH 7.5), 150mM

NaCl, 1mM EDTA, 1% SDS, and bacterial protease inhibitor cocktail (Sigma). After boiling, aliquots of the sample were applied to 15% SDS-PAGE gels. The recA proteins were detected with anti gyrase A antibodies (TopoGen). Densitometry was performed on IISP flat bed scanner and quantitated with Total lab 1.11 software. The expression of gyrase A protein was detected as band of molecular weight 97kDa (**Fig 1 (A)**). Quantification of each band by densitometric scanning showed significant decrease in the expression of gyrase A protein **(Fig 1 (B))** in the strain BL21 (DE3) that was transformed with pT7EGSEGyrA after 18 h of IPTG induction. No decrease in gyrase A protein expression was seen in BL21 (DE3) cells transformed with pT7EGSEGyrA when cells were collected at 5h (data not shown). Cells that were transformed with plasmid with the T7 promoterless construct too showed no change in the protein expression.

We suggest that the reduction of gyrase A protein might be due to possible binding of antisense molecules to gyrase A gene thereby inhibiting it and arresting the growth of bacterial system. Further studies should be carried out to verify the roles of EGS and its role as a potential therapeutic, as an effective intervention for infections.

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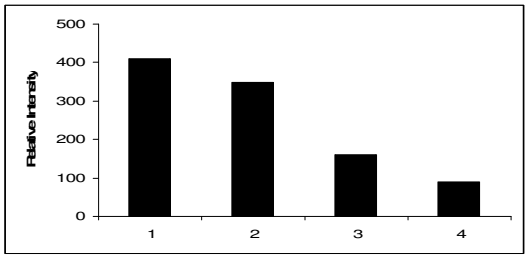

Fig 1. Representative of western blot analyses of gyrase A protein levels. (A) Western blot was performed with the protein that was isolated from the cells that were lysed after 18 h of induction. Lane 1 Cells transformed with T7 promoterless construct without IPTG induction. Lane 2 Cells transformed with T7 promoterless construct with IPTG induction. Lane 3 Cells with pT7EGyrEGS without IPTG induction. Lane 4 Cells with pT7EgyrEGS with IPTG induction. (B) Quantification of the intensity of bands in (A).