READTHROUGH OF PREMATURE STOP CODES IN DRUG METABOLIZING ENZYMES

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INTRODUCTION

Nonsense mutations are characterized by base changes that insert a premature stop codon, leading to truncated, non-functional proteins. Stop codons in pharmacogenes – genes that modulate response to pharmacological agents - have pharmacokinetic and/or pharmacodynamic consequences and therefore are important pharmacogenomic targets. Previous work from our group has shown that aminoglycoside antibiotics are capable of promoting readthrough of a stop codon in the *CYP2C19* gene (c.636 G>A, allele CYP2C19*3) and partially restore the expression and activity of the encoded drug metabolizing enzyme, *CYP2C19*. We are now extending this approach to a stop codon (c.802 C>T) in *CYP3A4*, which encodes the major route of drug metabolism in human liver, namely the enzyme CYP3A4.

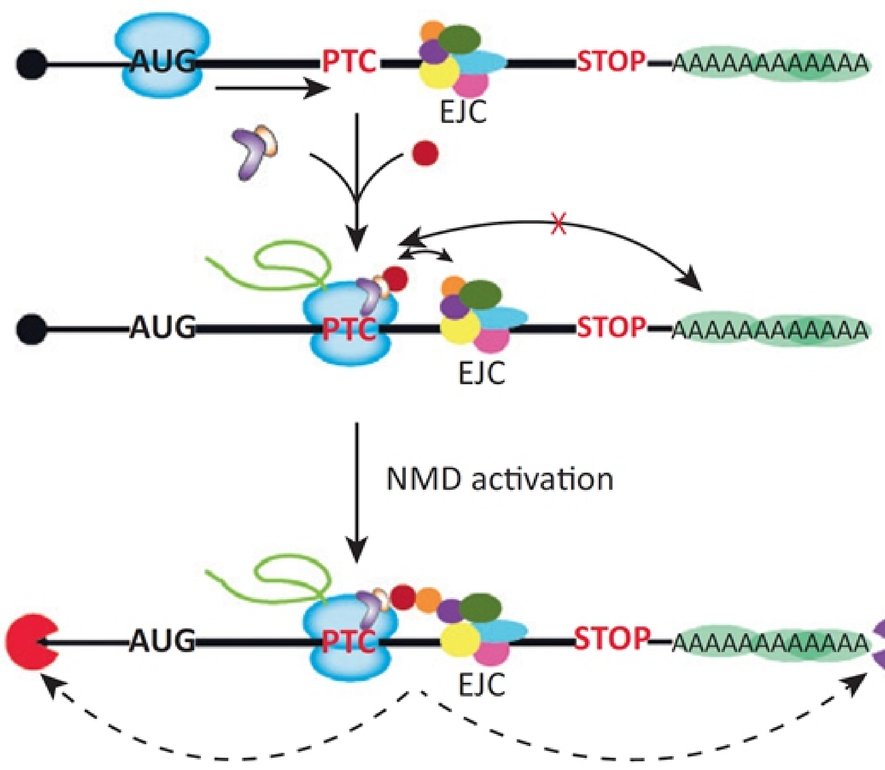


Figure 1: Schematic representation of the NMD via and premature stop codon. Adapted from Bidou et al., 2012.

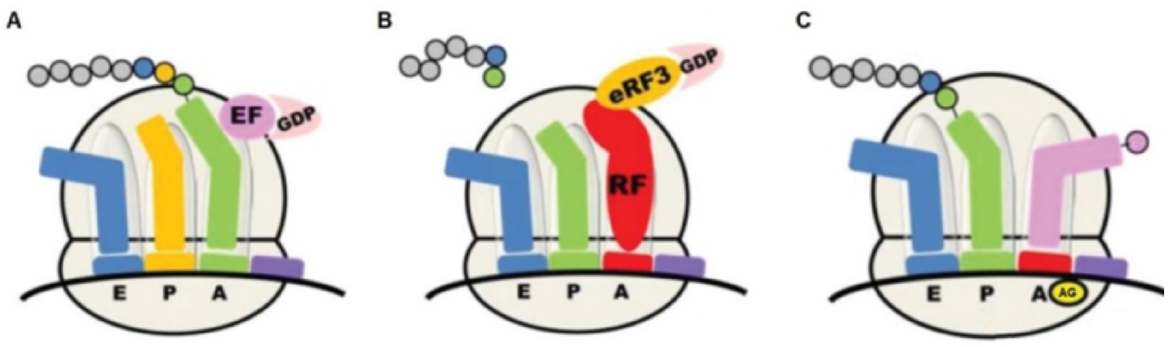


Figure 2: Schematic representation of aminoglycoside readthrough induction. A, translation of mRNA by ribosome; B, premature stop codon (red box) inducing termination of translation and originatting a truncated protein; C, aminoglycoside (yellow ellipse - AG) occupying minor subunit of the ribosome and leading to incorporation of a cognate amino acid to the nascent peptide. Adapted from Shalev & Baasov, 2014.

METHODS AND RESULTS

CYP3A4 wild-type sequence was generated by PCR and cloned into pCDNA3 vector. R268X CYP3A4 variant was generated by site-directed mutagenesis and will be subcloned into pQCXIH, in a fusion with EGFP. HeLa cells transfected with the constructions (wild type and R268X) will have expression levels evaluated by quantitative real-time PCR and immunoblotting. Cells will be treated with increasing concentrations of the aminoglycosides gentamicin and G418, and the expression of full-length protein will be examined by flow cytometry, immunoblotting and fluorescence microscopy. The functional activity of the translated protein will be evaluated by its enzymatic activity using P450-Glo™ CYP3A4 assay. The current status of the project will be presented.

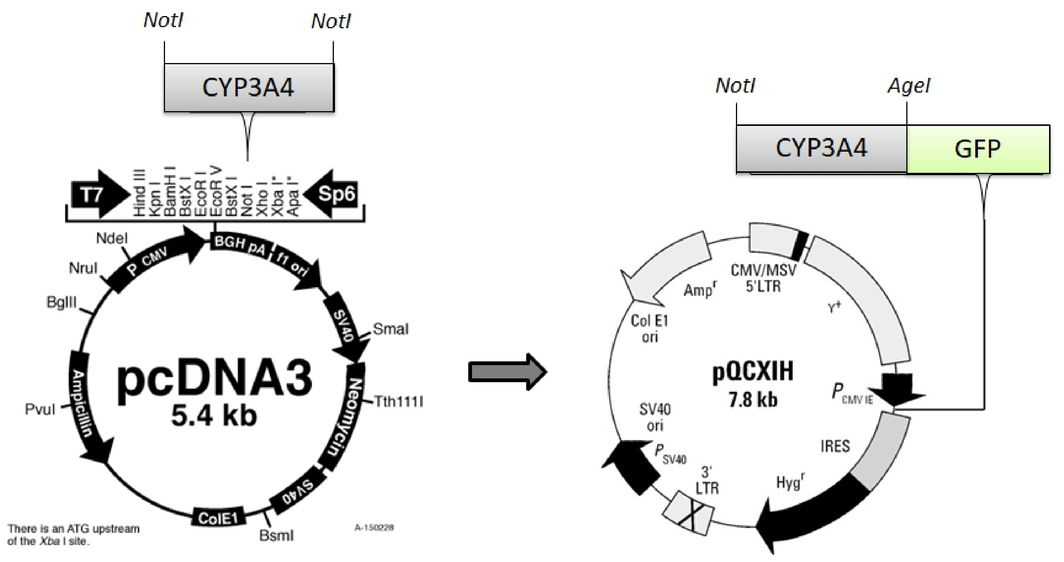


Figure 3: Map of the pcDNA3 vector with the coding sequence of *CYP3A4* used to transfer to retroviral vector pQCXIH, where it will be conjugated with GFP.

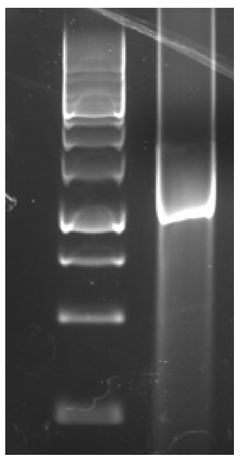


Figure 4: Electrophoresis analysis of PCR of *CYP3A4* sequence in agarose gel 1%.

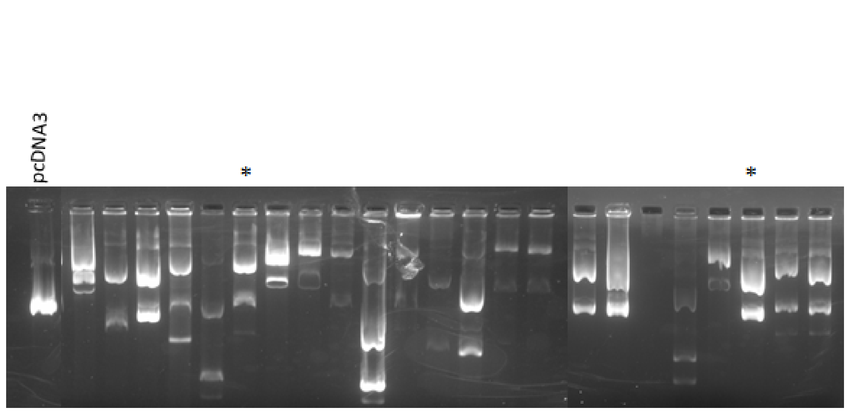
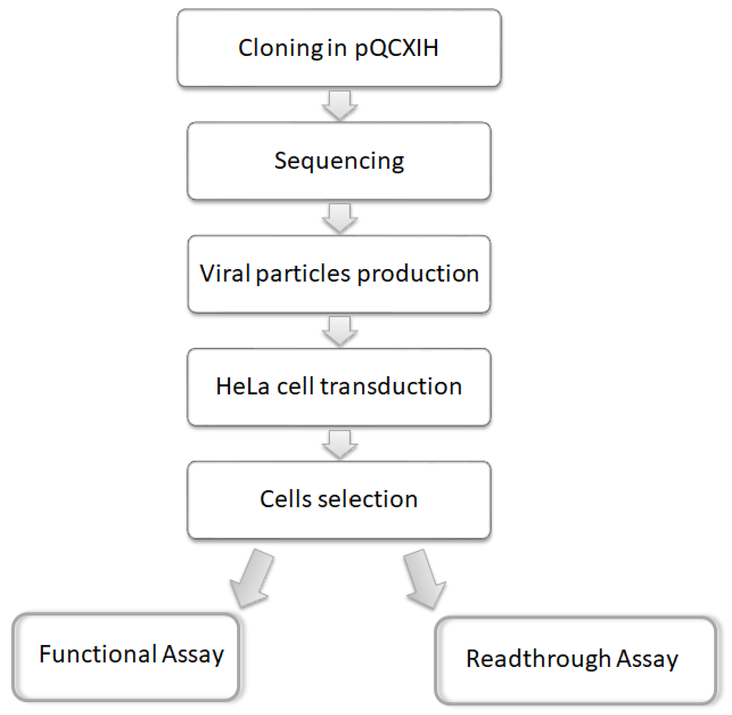


Figure 5: Electrophoresis analysis in agarose gel 1,5% of *CYP3A4* in DNA extracted from bacterial cells. The first column shows the pattern pQCXIH; * Represents positive sequences to *CYP3A4*.

CONCLUSION

We intend to provide evidence that aminoglycosides are capable of inducing read-through of the CYP3A4 c.802 C>T allele, yielding a functional full-chain protein and may be useful for restoring CYP3A4 enzymatic activity.



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