Analysis of cylooxygenase-1b (COX-1b) intron 1 length and protein expression

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Background: COX-1b differs from COX-1 in the retention of intron1. Retention of human intron1 leads to a frame-shift and a hypothetical truncation of the protein. Nevertheless, full-length COX-1b protein expression is detectable in human tissues and contributes to prostaglandin and thromboxane production in vitro. This study elucidates a potential mechanism of frame-shift repair and functional protein expression.

Methods: A COX-1b clone was N-terminally tagged with a His-Tag. COX-1b-His protein was overexpressed in HEK freestyle cells and purified. mRNA was extracted from human stomach and liver samples, confirmed to be free of genomic DNA and transcribed into cDNA. The region enframing intron1 was amplified and subcloned. On hundred and four independent clones were scanned for aberrations in intron1 length. RNA structure analysis utilised the algorithms RNAfold and pknotsRG.

Results: COX-1 overexpression and purification led to a single band in SDS page with a electrophoretic mobility of 72 kD, which could be detected by an anti-COX-1- and anti-His-antibody. All sequenced clones comprised an intron1 sequence of 94 bp, theoretically leading to a frame-shift and protein truncation. RNA structure algorithms predicted hypothetical slippery sequences in combination with thermostable pseudoknot structures, which is a premise for ribosomal frame-shifting.

Conclusions: Full-length COX-1b protein can be overexpressed in a human cell line and results in the expected protein size comprising the signalling peptide, the intron1 insertion and complete COX-1 protein sequence. Retention of the signalling peptide was proved. We found no evidence for RNA-editing, a postulated frame shift repair mechanism for COX-1b. RNA structure analysis provides implications for +1 ribosomal frame-shifting which would revert the sequence into the open reading frame, resulting in full-length COX-1b protein production.