A GFP-based assay for monitoring post-transcriptional regulation of ARE-mRNA turnover

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Interleukin-3 (IL3) mRNA is intrinsically labile due to the presence of a destabilizing AU-rich element (ARE) that targets the transcript for rapid degradation. We review our experience with a sensitive reporter system where changes in IL3 mRNA stability are translated into increased/decreased green fluorescent protein (GFP) signals. A GFP reporter gene was fused to the full-length IL3 3'UTR containing the ARE motif that responds to regulatory signals that control transcript stability. The reporter system was tested against known IL3 mRNA stabilizing/destabilizing agents either through pharmacological treatment, siRNA knock-down of components of the decay machinery, mutation of the ARE motif, or in tumour lines harbouring stable IL3 mRNA. In all cases, the reporter transcript responds in an identical fashion to the endogenous IL3 message thereby verifying the fidelity of the system. This reporter system allows screening and identification of novel ARE-mRNA stabilizing compounds, or the isolation of mutants defective in ARE-mRNA turnover. We also report preliminary attempts to modify the system for high-throughput screening of an extensive compound library. The simplicity and effectiveness of this screen makes it ideal for screening of modulators of clinically important ARE-bearing transcripts such as TNFalpha, VEGF, the interferons and other cytokines.

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