Transcriptional regulation of the mouse Tissue Inhibitor of Metalloproteinase (*Timp1*) gene is mediated by transcriptional attenuation in intron 1.

Caroline J Pennington, Ian M. Clark, Dylan R. Edwards

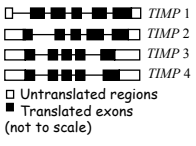
BioMedical Research Centre, University of East Anglia, UK

Introduction

Tissue inhibitors of metalloproteinases (*TIMP*s) are a family of four multifunctional proteins with a broad range of biological activities. These include the inhibition of metalloproteinase activity and the regulation of cell proliferation, apoptosis, angiogenic and inflammatory responses.

Elevated mRNA expression of *TIMP* family members correlates with malignancy and clinical outcome in many human cancer types; however, a protective role for *TIMP*s has also been observed in various mouse models of human cancer.

Mammalian *TIMP2*, *TIMP3* and *TIMP4* genes contain 5 exons, *TIMP1* is unique in that it has an additional short first exon which is transcribed but not translated.



Objective

Previous work has shown that the regulation of *TIMP1* expression occurs at the level of transcription.

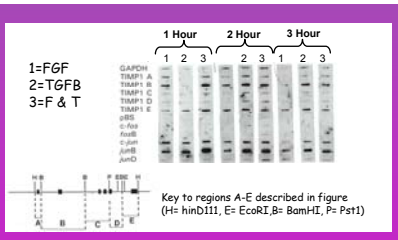
In this study we examine the function of the first intron in regulating expression of murine *TIMP1*. We describe a novel method of using TaqMan® Real Time PCR technology to quantify levels of transcription throughout the gene with particular attention to intron 1.

Methods

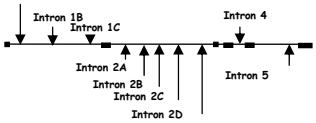
Mouse fibroblast cells (C3H/10T$^{1}/_{2}$) were stimulated with PMA,TGFB, FGF or TGFB and FGF in combination for 1, 2 or 3 hours prior to cell nuclei isolation and incubation with radiolabels (nuclear run-ons), or total RNA extraction, reverse transcription and Real Time PCR amplification.

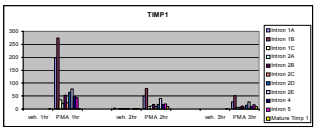
Results

Using nuclear run on assays (to identify genes that are being transcribed at a certain point in time in a cell) we show that *TIMP* 1 transcription is not uniform across the gene. Levels of transcription prior to the mid region of intron 2 are higher than levels following that point.

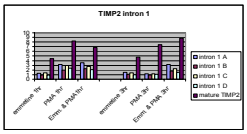


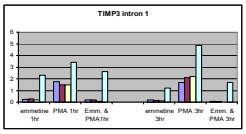
TaqMan® primers and Universal Library Probes (Roche) were designed to amplify intronic regions throughout the murine *TIMP* 1 gene.





Analysis of Real Time PCR revealed a marked decrease in gene transcription of intron 1 after +522 (the position of probe 1B). This corresponds with nuclear run on data but allows more precise positioning of the region of transcriptional attenuation.





The *TIMP* 1, 1st intron profile contrasts with transcription profiles of intron 1 in both *TIMP* 2 and 3. Amplification of regions throughout the first introns of these *TIMP*s do not alter significantly.

Timp 1 intron 1

GTAAGTGGGAGCCGGGGTGTCCTGGCAGAACTGGGGCTTGCCTGGAGCCTGTGTTGGTGCGGCTCATGCAGCCCTTGCCAGACTAACTGCTCACGTGGCTTGCAGGT
ATCAGGATCCCTAGTTAGCTAAAGAGGAATATAACGGGAGAGGGCCTGGAATGATCGTCCCAAGCTGGACTCTCCAGACTCCTTCATGCCTTTGCTAAGTGCTCCAAAC
CCACAAGCCTTTAATACCTTGAAGACTTCCCAACTTGCCTGTCCCTCAATCCTTATGGTTCCTGGTAACTCCCCTGTTCT...TTTGCCATGTCTGGTTCATGTGAACTTCAA
CAGTTCTTCTGCCTACAAATTCCTCAAGCATTACCATTGTCGTAAACCTCCAAATTCCCCAAGACCCTTAACCCCCATTTTCTCCAGCTCCCTAAAACCCCAAACCTGTTC
TAACCCCCAATATCTCTGAAATCCTCAGCCCATTATATTTTAATACCCTAATTCTCCATATATATTTCTCCAGTTCCCAACTCCAAATTCATCACTACACAAATGTCAG
ATTTTCCCAATTCTGTTAAATTCTCAACGTTCTCCGCTCCCTTTATTCACCAAACTGCCACAACTCCATTCTCTAACTCTAAAGAGTCTCCCTAGTAAATCTCCCTAGTGACT
AATTGGCCCATGTTAAGCCGGTTGGTCTGGGAACCTATTAACACCTACTCTTTACCAG

Analysis using 3'-RACE further indicates the presence of primary transcripts that terminate at various sites towards the 3'-end of intron-1. Green sequence = TaqMan amplicon, # = termination site.

Conclusions and future work

These data indicate that *TIMP 1*, but not other *TIMP* family members, is subject to control by transcriptional attenuation within its distinctive intron 1 region. Further work will concentrate on the mechanism of transcription attenuation and the possible role played by chromatin remodeling.