# Investigating the Variability of Cyclooxygenase-1

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## INTRODUCTION

Cyclooxygenase-1 (PTGS-1) is a potent mediator of inflammation. It mediates the conversion of arachidonic acid into $PGG_2$ and $PGH_2$. These products can then be used by other downstream processes leading to either acute or chronic inflammation.

We hypothesize that the deciding factor between acute and chronic inflammation depends on alternative splicing of the mRNA transcript. Currently, there are around 7 known splice variants of PTGS-1. These splice variants are abundant in cancerous cells, and chronic inflammation is expected in cancer. One variant of particular interest is found in rat epithelial cells. This variant is unique because it retains only 222bp from an intron that is over 6600bp long.

Recent evidence from our lab suggests that the 5' UTR of any of these variants may be differentially spliced and an alternate promoter may be used to regulate expression. This may provide a basis for the transition between acute and chronic inflammation.

## MATERIALS AND METHODS

*Cell Culture* – The human myeloid cell lines HL-60, U937 and THP-1 were cultured in RPMI 1640 with 10% heat inactivated fetal bovine serum (Gibco), and 10 mM L-glutamine (Gibco). Cells were cultured in a 5% $CO_2$ atmosphere at 37°C. Log-phase cells were differentiated with the following techniques. U937 and THP-1 cells were treated with 10 ng/mL TPA (Sigma-Aldrich) for 24 hours prior to RNA extraction. HL-60 cells were treated with 1.25% DMSO (Sigma-Aldrich) 7 days prior to RNA extraction. Cell counts were performed with a Nexelon K2 cellometer or by hemocytometer.

*RNA Extraction* – RNA was extracted from approximately $1x10^7$ cells using the RNAqueous-4PCR Kit (Ambion, ThermoFisher). We employed the optional DNAse I treatment.

*RT-PCR and Amplification* – cDNA was created using the SuperScript IV First-Strand Synthesis System using poly dTTP primers. cDNA was then amplified using a forward primer for intron 2 of PTGS1 and a reverse primer near the stop codon of exon 11 through 40 cycles in a thermocycler.

*Gel Electrophoresis* – PCR amplified cDNA samples were loaded into a 1% TAE agarose gel (Invitrogen UltraPure Agarose), and electrophoresed for approximately 4 hours at 97 V. Bands of interest were cut from the gel and DNA was extracted with the PureLink Quick Gel Extraction Kit (Invitrogen, ThermoFisher).

*Sequencing* – Resulting purified DNA was then TOPO cloned (Invitrogen), prepped and sent for sanger sequencing at the BYU DNA Sequencing Center, or the Nevada Genomics Center.

## RESULTS

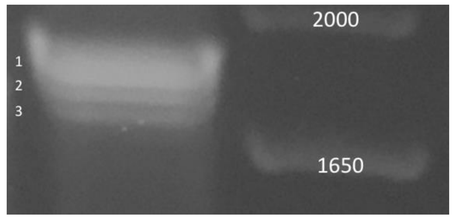


**Figure 1:** Gel electrophoresis of PCR products using cDNA from unstimulated HL-60 leukemia cells with partial intron 2 forward primers.

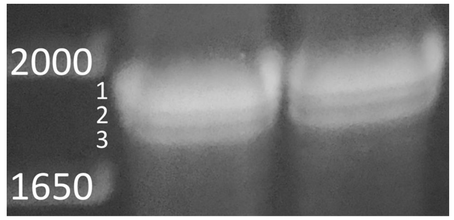


**Figure 2:** Gel electrophoresis of PCR products using cDNA from TPA-stimulated THP-1 leukemia cells with partial intron 2 forward primers.

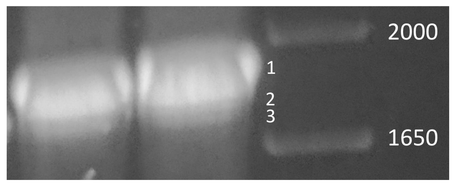


**Figure 3:** Gel electrophoresis of PCR products using cDNA from unstimulated/TPA stimulated U937 leukemia cells with partial intron 2 forward primers.


**Figure 4:** Sequence data of excised bands from **Fig. 1** correspond to the following splice variants.

## RESULTS (CONT.)

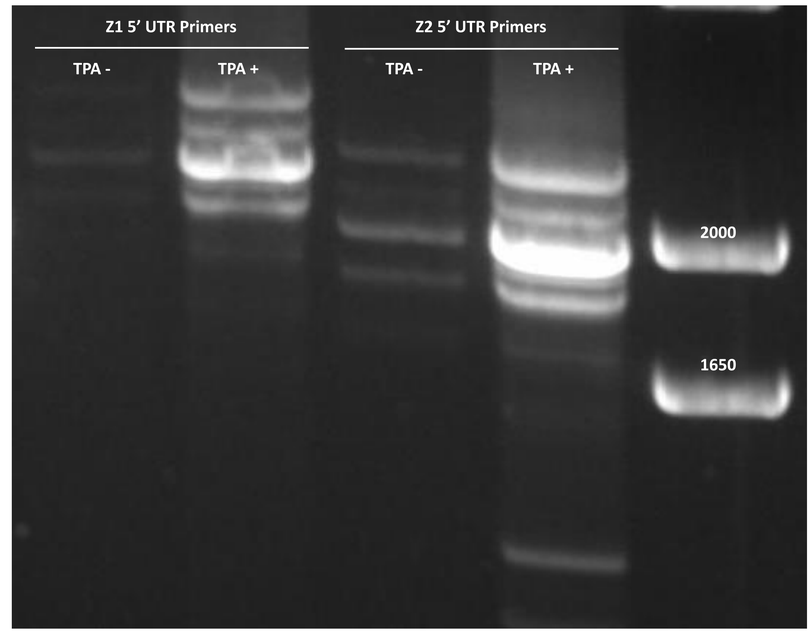


**Figure 5:** Gel electrophoresis of PCR products using cDNA from THP-1 with variable 5' UTR forward primers.

## CONCLUSION

Alternative splicing of the 5' end of PTGS-1 mRNA may play a role in acute vs. chronic inflammation. However, with cDNA from unstimulated cells resulting in positive amplification of intron 2 retention variants, but not full length PTGS1, it also appears that the intron 2 retention variants may also play a part in regulation. Data suggests that variants containing exon 1 may primarily be responsible for acute inflammation.

## FUTURE DIRECTIONS

The 5' UTR of these splice variants still needs to be fully characterized. Additionally we will use multiplexed TaqMan RT-qPCR and multiplexed fluorescent probe northern blot to confirm the presence of variants of interest.

Additionally, we need to clone the splice variants, and test for PTGS-1 activity, and consequently, NSAID inhibition of PTGS-1. Ultimately, all these experiments will be validated in whole blood.

## ACKNOWLEDGEMENTS

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