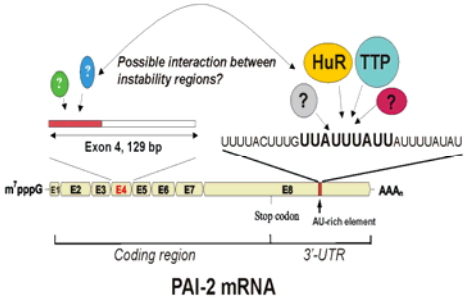
Project 1080

Is the expression of the plasminogen activator inhibitor type 2 gene subject to Post-transcriptional regulation?

Supervisors **Dr. Stan Stasinopoulos and A/Prof Robert L. Medcalf**
E-mail Stanley.Stasinopoulos@med.monash.edu.au
Robert.Medcalf@med.monash.edu.au

Our laboratory is interested in understanding the mechanisms that govern gene expression of members the plasminogen activating (PA) system. The end product of this system is a powerful protease called plasmin that can degrade blood clots and a number of other proteins within the extracellular matrix. The generation of plasmin from plasminogen is mediated by the plasminogen activators: tissue-type plasminogen activator (t-PA) and urokinase (u-PA). The activity of the two plasminogen activators is regulated by specific inhibitors, known as PAI-1 (that inhibits t-PA and u-PA) and PAI-2 (that preferentially inhibits u-PA).



PAI-2 mRNA

PAI-2 is an unusual protease inhibitor as the majority of PAI-2 is located intracellularly. In the last few years novel intracellular roles have been attributed to PAI-2, including as a survival gene in macrophages following bacterial infection. PAI-2 has also been shown to inhibit apoptosis and to modulate activity of a tumour suppressor protein. PAI-2 also enhances HIV replication while HIV infection increases PAI-2 expression. PAI-2 is also a highly regulated gene, with its level of expression being massively increased in cells under some conditions.

We have been studying the biology of PAI-2 with particular emphasis on gene regulation. We have shown that the fold induction of PAI-2 mRNA levels is dramatically stimulated by tumour promoting agents (e.g. phorbol esters) and cytokines (eg TNFα). Previous reports have suggested that the increase in PAI-2 mRNA is not only transcriptional, but also includes post-transcriptional events. Post transcriptional regulation is often controlled by sequences located in the 3' untranslated region (3'-UTR). To date we have mapped and identified three discrete sequence elements within a 120 nt region in the 3'-UTR of the PAI-2 transcript, and one coding sequence element within Exon 4, that markedly influence PAI-2 mRNA stability. Moreover, we have a number of candidate RNA binding proteins that could be involved in PAI-2 mRNA decay/stabilisation *via* the 3'UTR. The current hypothesis suggests that these 3' UTR-localised elements can modulate PAI-2 mRNA decay (e.g. either increase or decrease mRNA stability), and possibly even translational initiation, depending on the physiological status of the cell.

This Honours project will assess the contribution of these elements to the overall control of PAI-2 mRNA decay *in vivo* using human Fibrosarcoma (fibroblastic sarcoma) and U937 (myeloid leukaemia) model cell lines. This project will also use RNA binding assays to determine whether proteins selectively bind to one or more of these regions and if this is altered when cells are stimulated with agents [e.g. cytokines (TNF), carcinogens (phorbol ester) and glucocorticoids (dexamethasone)], that regulate PAI-2 gene expression. This work will provide a basis for a proteomic approach to identify these RNA binding proteins, and will not only add to the understanding of PAI-2 biology, but will also contribute to the broader, and increasingly important, field of post transcriptional gene expression.