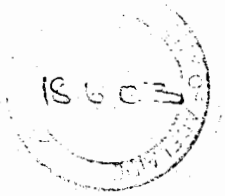

INVESTIGATING THE ROLE OF ARNT ISOFORMS AS bHLH/PAS TRANSCRIPTIONAL REGULATORS

This thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in the Department of Molecular Biosciences (Biochemistry), University of Adelaide

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THESIS SUMMARY

The basic helix-loop-helix PAS transcription factor ARNT functions as an obligate partner protein for signal regulated bHLH/PAS factors such as the Dioxin Receptor (DR) and Hypoxia Inducible Factors (HIF-α). The DR and HIF-α proteins respond to the respective environmental stimuli of xenobiotic exposure or low oxygen tension by forming active heterodimeric complexes with ARNT. Given the potential for ARNT to act as a general dimerisation partner for the emerging bHLH/PAS factor family, there has been some speculation as to the physiological role of ARNT in the absence of DR ligand or hypoxia.

The likelihood of further transcriptional regulatory roles is supported by evidence that ARNT can recognise the CACGTG E-box element as a homodimer *in vitro* and *in vivo*, the constitutive nuclear localisation of ARNT, the widespread expression of ARNT in mammalian tissues and the presence of a strong transactivation domain within the C-terminus of ARNT. The identification of an alternatively spliced form of the ARNT gene, known as Alt ARNT, provides additional complexity to the role of ARNT in the cell.

This thesis examines the possibility of alternative roles for ARNT in the cell. A search for downstream target genes of ARNT was performed utilising the suppression subtractive hybridisation-PCR method. A subtracted library was generated using cDNA from Hepa 1c1c7 cells stably overexpressing a dominant negative form of ARNT lacking the basic DNA-binding domain, known as ARNTΔb. The subtracted library was screened to identify potential differentially expressed cDNAs. Subsequent Northern blot analysis revealed one of the clones identified during the screen was differentially expressed between the ARNTΔb Hepa cells and Hepa 1c1c7 cells stably containing a blank expression vector. It is unlikely, however, to be a direct target of ARNT as it was expressed more highly in both ARNT and ARNTΔb overexpressing cells compared to control Hepa 1c1c7 cells and contained no known ARNT binding sites within 712 bp of available promoter sequence. An additional differentially

expressed gene, CD47, was identified using a microarray data set obtained from another laboratory project, though the role of ARNT in regulation of CD47 expression remains unclear. These results suggest an alternative approach, perhaps detailed microarray analysis, is required to investigate novel roles for ARNT as a transcription factor.

This thesis also sought to investigate the function of Alt ARNT as a transcriptional regulator. Work herein describes the differential regulation of Alt ARNT and ARNT by Protein Kinase CKII. Phosphorylation was shown to have an inhibitory effect on DNA-binding to the E-box as homodimers. In contrast, DNA-binding by Alt ARNT/Dioxin Receptor heterodimers to the xenobiotic response element was not inhibited by phosphorylation. Together with the co-expression of ARNT and Alt ARNT found in all cell types tested, these results are supportive of alternative roles for these two ARNT isoforms existing within the cell.