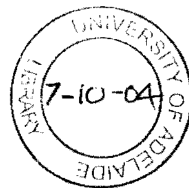


# Regulation of Gene Expression of the 25-Hydroxyvitamin D 1α-Hydroxylase (CYP27B1) Promoter: Study of A Transgenic Mouse Model

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A thesis submitted for the degree of

***Doctor of Philosophy***

to

The University of Adelaide

March 2004

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*Abstract*

The enzyme 25-hydroxyvitamin D 1α-hydroxylase or CYP27B1 is the key enzyme in the two-step activation process by which vitamin D is converted to its biologically active form 1,25-dihydroxyvitamin D (1,25D). The actions of a number of regulators on the renal CYP27B1 enzyme activity have been recognized for some years, although the underlying molecular mechanisms remain largely unknown and the DNA regions involved in the *in vivo* regulation of gene expression by these factors have not been delineated as yet.

In order to identify the regulatory regions through which these factors control CYP27B1 expression in the kidney *in vivo* and to study the spatial and temporal expression of the CYP27B1 gene during development, a transgenic mouse model was established. This model was developed using pro-nuclear injection of a DNA construct containing the firefly luciferase reporter gene under the control of the 1541 bp region of the human CYP27B1 promoter.

Following pro-nuclear injection, three transgenic founders were obtained and bred to establish three independent transgenic lines. In all three lines, a very similar expression pattern of the luciferase reporter gene was detected. High levels of luciferase activity were detected in the kidney, brain, testis, skin and bone. Lower levels of luciferase activity were detected in heart, lung, liver, distal small intestine, skeletal muscle and spleen extracts. No reporter gene expression could be detected in the proximal small intestine.

This animal model was used to identify the ability of the 1541 bp promoter region of the CYP27B1 gene to respond in the kidney to a number of physiological challenges including dietary calcium, vitamin D and the immunomodulator LPS. In addition, the temporal expression of the reporter gene was studied by sacrificing animals at 6 different time points (2, 4, 6, 8, 12 and 64 weeks of age). The functionality of the CYP27B1 promoter was verified by comparing the regulation of the expression of the reporter gene with that of the endogenous CYP27B1 gene. The expression of endogenous CYP27B1 mRNA levels was therefore determined using Real-Time RT-PCR.

The expression of the reporter gene and the endogenous CYP27B1 mRNA levels in the kidney were increased during early development (2 week old animals) and fell with increasing age. Reporter gene expression and CYP27B1 mRNA levels were down-regulated in response to increasing amounts of dietary calcium in a dose-dependent manner. Vitamin D-deficiency resulted in an increase in both the reporter gene and CYP27B1 expression. However, the increase in CYP27B1 mRNA levels was substantially higher than the increase in reporter gene expression, suggesting that other regulatory elements are required to maximize the effect of vitamin D-deficiency. LPS administration did not affect the expression of either luciferase or the endogenous CYP27B1 gene in the kidney.

Immunohistochemistry was used to identify the cell-specific location of the luciferase and the endogenous CYP27B1 protein in the kidney in kidney sections of vitamin D-deficient animals. Both luciferase protein and the endogenous CYP27B1 protein were identified in the proximal tubular cells of the kidney.

The regulation of the expression of the reporter gene was also studied in the transgenic mouse model in a number of extra-renal tissues that have been shown to express CYP27B1 and to be responsive to 1,25D. These tissues include heart, liver, lung, femora, bone marrow, skeletal muscle, testis, skin, brain, spleen and proximal and distal small intestine. Although in most tissues, the expression of luciferase was highest in the 2 week old animals and fell with increasing age, in the testis, the expression levels were low in the developing animals and increased with increasing age.

No physiological significant effects were detected in any of the extra-renal tissues examined in response to dietary calcium and vitamin D, suggesting that these factors control CYP27B1 expression in a kidney-specific manner. In addition, no physiologically significant effect of the LPS administration could be detected in these tissues.

Future studies employing transgenic animals which express transgenic constructs containing both the CYP27B1 promoter and upstream and/or intronic sequences are required to identify the factors that regulate CYP27B1 expression in the different tissues and to delineate the DNA regulatory regions through which these factors exert their effects *in vivo.*