# REGULATION OF THE RAT 25-HYDROXYVITAMIN $D_3$ 24-HYDROXYLASE GENE PROMOTER BY $1,25(OH)_2D_3$



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**Thesis Summary**

The secosteroid 1,25-dihydroxyvitamin $D_3$ ($1,25(OH)_2D_3$) is an important regulator of calcium homeostasis, cellular differentiation and proliferation. The nuclear actions of $1,25(OH)_2D_3$ are mediated through the intracellular vitamin D receptor (VDR), a member of the nuclear receptor superfamily of transcription factors. The metabolic inactivation of $1,25(OH)_2D_3$ through the C-24 oxidation pathway is initiated by a mitochondrial cytochrome P450 enzyme (CYP24). The action of CYP24 initiates the conversion of $1,25(OH)_2D_3$ to water soluble metabolites. It has been well characterised that $1,25(OH)_2D_3$ acts to stimulate CYP24 gene expression in a negative feedback loop.

The work undertaken in this thesis was aimed at understanding at the transcriptional level how $1,25(OH)_2D_3$ up-regulates CYP24 gene expression. Previous studies with the rat CYP24 promoter have identified three putative vitamin D responsive elements (VDREs), termed VDRE-1,2 and 3 in the first 298 bp of the CYP24 promoter region. To elucidate how these putative VDREs function in the context of the native promoter environment, the putative VDREs were mutated either individually or in combination by site-directed mutagenesis. The mutant promoter constructs fused to the luciferase reporter gene were then examined in transient transfection assays in the kidney and bone cell lines COS-1, JTC-12 and ROS 17/2.8 in the presence or absence of $1,25(OH)_2D_3$. These studies have demonstrated that VDRE-1 and VDRE-2 are solely responsible for the $1,25(OH)_2D_3$ mediated response. VDRE-1 contributes 6-fold and VDRE-2 3-fold to the overall induction of 18-fold for the native 298 bp promoter construct, thus demonstrating a 2-fold synergistic relationship between these VDREs. VDRE-3, however was unresponsive to $1,25(OH)_2D_3$ even when VDRE-1 and 2 were mutated.

The three VDREs were also investigated at the protein-DNA level by gel-shift analysis. VDRE-1 & 2 were shown to bind a protein complex of approximately the same mobility as that which binds to the well characterised VDRE of mouse osteopontin (mSPP-1). The components of this complex were elucidated by the use of supershifting and competitive monoclonal antibodies and demonstrated to be VDR and its partner factor retinoid-X-receptor (RXR). Competition analysis revealed that VDRE-2 binds the VDR-RXR heterodimeric complex with a higher affinity than VDRE-1; this finding is in contrast to the transient transfection data where VDRE-1 has greater transcriptional activity. This raises the possibility of other transcription factors being involved in the $1,25(OH)_2D_3$ response by enhancing VDRE-1 activity.

The functionality of the characterised VDREs at more physiologically relevant concentrations of $1,25(OH)_2D_3$ was determined. In COS-1 cells co-transfected with VDR, it was found that both VDREs were functional, with synergism observed over a wide range of $1,25(OH)_2D_3$ concentrations ($10^{-7}$-$10^{-11}$ M). At lower concentrations only VDRE-1 was responsive to $1,25(OH)_2D_3$ and similar results were obtained in ROS 17/2.8 cells which express significant levels of endogenous VDR, thus demonstrating that the synergism observed may have important physiological consequences. In JTC-12 cells induction was only observed at very high concentrations of $1,25(OH)_2D_3$ and may be a reflection of low VDR levels.

In summary, the results establish a so far unique synergistic relationship between two VDREs which is maintained at physiological concentrations of $1,25\text{-}(OH)_2D_3$ with VDRE-1 the major contributor to induction, particularly at low hormone concentrations.