Isolation and Characterisation of CRTR-1 and altCP2: Negative Regulators of CP2 Transcription Factor Family Activity.

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SUMMARY

Mouse CP2 is the founding member of a group of highly conserved proteins in mouse, human, chicken and *Drosophila* collectively referred to as the CP2 family of transcription factors. CP2 was originally identified in murine erythroleukemia (MEL) cell nuclear extracts as an activator of the mouse α-globin gene, binding a promoter element overlapping the CCAAT box. In addition to CP2, the family includes mouse NF2d9, human LBP-1c and LBP1a, homologues of mouse CP2 and NF2d9 respectively, LBP-9, chicken CP2 and *Drosophila melanogaster* CP2. Mammalian CP2-related proteins have generally been shown to be activators of transcription and expressed ubiquitously. There is little knowledge of factors that regulate their activity.

This thesis describes CRTR-1, a novel member of the CP2 family, originally identified as a transcript differentially expressed between mouse ES and early primitive ectoderm-like (EPL) cells *in vitro*. *In vivo* expression analysis showed CRTR-1 to be expressed by the pluripotent inner cell mass cells of the blastocyst, but not in the later pluripotent cells of the primitive ectoderm. Analysis during later stages of development and in the adult mouse showed CRTR-1 expression to be developmentally and spatially regulated. Greatest levels of CRTR-1 expression were detected in the embryonic and adult kidneys with CRTR-1 specifically expressed in the branching ureteric buds of the developing kidney with expression becoming restricted to the epithelial lining of the distal convoluted tubules during later kidney development and in the adult mouse. These sites of CRTR-1 expression suggest distinct biological roles for CRTR-1 function in the maintenance of pluripotency in the early stage embryo and in the development and function of the kidney.

Conservation in nucleotide and amino acid sequence defined CRTR-1 as a novel member of the mouse CP2 family of transcription factors. Consistent with this,

CRTR-1 was shown to interact with all other members of the mouse CP2 family, forming protein complexes competent to bind a CP2 family consensus DNA response element. However, unlike other CP2 family members, CRTR-1 was shown to act as a transcriptional repressor with the ability to repress transcription localised to a novel 52 amino acid N-terminal repression domain. Furthermore, the ability of CRTR-1 to repress transcription was shown to be dominant over CP2 mediated transcriptional activation. CRTR-1 is therefore distinct from other family members in two respects, CRTR-1 expression is spatially and temporally regulated and CRTR-1 acts as a dominant transcriptional repressor of CP2 family mediated transcriptional activation.

This thesis also describes the identification, isolation and functional characterisation of an alternatively spliced isoform of CP2, altCP2. Similar to CRTR-1, altCP2 appears to be differentially expressed and was demonstrated to act as a dominant repressor of CP2 family mediated transcriptional activation by formation of heteromultimers with other CP2 family proteins that cannot bind DNA. Together, altCP2 and CRTR-1 provide mechanisms to achieve spatially and temporally regulated activity of ubiquitously expressed CP2 family transcriptional activators.

Possible mechanisms regulating the cellular localization and transcriptional regulatory ability of CP2 family members were investigated by identification of non-related binding proteins. Yeast-2-hybrid analysis identified Ubc9, PIAS1 and FKBP4 as CRTR-1 binding proteins. Ubc9 and PIAS1 function as regulators of post-translational modification by sumoylation. These have been shown to regulate the cellular localization and transcriptional regulation of other transcription factors, and were shown here to interact with other mouse CP2 family members. In contrast the immunophilin FKBP4 was determined to be a CRTR-1 specific binding protein suggesting that alternate mechanisms regulate CRTR-1 and other family members.

Identification of CP2 family member specific binding proteins suggest mechanisms for independent regulation of CP2 family members through distinct cellular pathways.

Finally, identification of the sumoylation enhancer PIAS3 and transcription factors Rex1 and YY1 as CRTR-1 binding proteins provide further mechanisms for the regulation of CRTR-1 activity through the control of cellular localization, transcriptional regulation and promoter specificity and together suggest mechanisms for the regulation of CRTR-1 activity through signaling pathways important for pluripotence and kidney development.