The hormonal regulation of carbamoylphosphate synthetase I expression
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Scope
**SCOPE**

Glucocorticoids exert their effects by binding to and activating the glucocorticoid receptor. Subsequently, the glucocorticoid receptor can activate or repress the expression of target genes by direct binding to the regulatory regions of these genes, or by indirect mechanisms. Although the glucocorticoid receptor is expressed throughout the body, many of its target genes are not. To this end, binding sites for the glucocorticoid receptor are clustered together with other transcription factor-binding sites in so-called glucocorticoid-responsive units (GRUs). All transcription factors acting in such a GRU, need to bind their response element before gene activation can take place. Carbamoyl/phosphate synthetase I (CPS) serves as a paradigm to study glucocorticoid-dependent regulation of gene expression in the liver. A 469-basepair enhancer, located 6.3 kb upstream of the transcription-start site, confers liver-specific, hormone-inducible periportal expression upon the gene. Within this enhancer, a glucocorticoid-responsive unit is located that harbours 50% of the total glucocorticoid-induced activity. Using *in vitro* footprinting studies, it was shown that this region contains binding sites for the ubiquitously expressed glucocorticoid receptor (GR), the liver-enriched transcription factors FoxA and C/EBP, and an unknown protein P3.

The scope of this thesis is to determine how the regulatory elements of the CPS gene act together to mediate transcription. Chapter 1 reviews the current state of the art in glucocorticoid signalling mediated by simple GREs and GRUs. In chapter 2, we describe two approaches to remove multiplicative variation between transfection data. In the first method, we use the statistical package SPSS to determine between-session factors, whereas the second approach uses the factor-correction program. In chapter 3, we investigate the architectural composition of the glucocorticoid-responsive unit of the CPS gene in mediating the glucocorticoid response. We found that the function of the GRU is dependent on the binding of four transcription factors. Chapter 4, describes the dependency of the GRU on sequences proximal to the promoter, which may establish a physical link between the distally located GRU and the promoter. In chapter 5, we focus on the 5' region of the 469-basepairs enhancer. Within this region, we identify a CRU that is the sole mediator of the cyclicAMP response, but in addition, is also a mediator of the glucocorticoid response.