7 Abstract

Spermatogenesis is a process in which diploid precursor cells develop into mature haploid spermatozoa. The molecular mechanisms of its regulation are not well understood. The cDNA of the nuclear orphan receptor GCNF has been isolated from mouse. Its expression profile was found to be germ cell-specific. A possible function of GCNF during germ cell development is conceivable. Therefore, GCNF is considered a possible target for fertility control.

This work describes the isolation and characterization of human GCNF. The human full-length GCNF cDNA, isolated from a human Testis cDNA-library, showed a high level of identity with the mouse GCNF sequence (89.1% and 98.3% nucleotid sequence and amino acid sequence identity, respectively). The expression profile of hGCNF mRNA, investigated by Northern blot analysis, revealed an almost exclusive expression in testis. In order to investigate the expression of hGCNF in testis in more detail, hGCNF fragments were expressed in *E.coli*, purified and used for generation of antibodies. By using these antibodies, it could be shown that GCNF is expressed in human, monkey, dog and mouse testis exclusively in post-meiotic round and elongated spermatocytes. To elucidate the intracellular localisation a hGCNF-EGFP fusion protein was expressed in HeLa cells. A predominant nuclear and weak cytoplasmatic expression was detected. This result was further supported by the immunohistochemical detection of full length hGCNF expressed in HeLa cells. Furthermore the function of GCNF was investigated by transactivation assays. It could be shown that GCNF acts as a repressor of transcription on reportergenes. By using deletion mutants, the repressor domain could be mapped to the ligand-binding domain, which possibly contains two repressor domains. The interaction of GCNF with the corepressor NCoR was investigated using the yeast and mammalian two hybrid system and GST-pull down experiments. The results of all three systems confirmed the interaction of GCNF with NCoR. Deletion analyses using the GST-pull down assay allowed to map the interaction region of GCNF with NCoR to three regions within the ligand-binding domain.