

## REVIEW

Holger Greschik · Roland Schüle

**Germ cell nuclear factor: an orphan receptor with unexpected properties**

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**Abstract** The orphan receptor germ cell nuclear factor (GCNF) is a member of the superfamily of nuclear receptors. During embryogenesis GCNF expression is restricted to the developing nervous system, whereas in the adult the receptor is also expressed during specific stages in maturing germ cells of the ovary and testis. Therefore GCNF may participate in the regulation of neurogenesis and re-

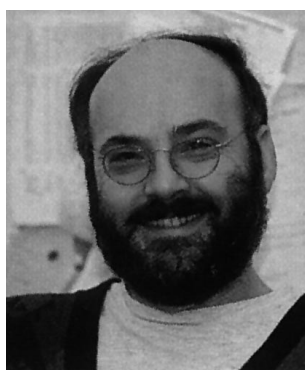
productive functions. Binding of GCNF to the consensus element TCA[AG(G/T)TCA]<sub>2</sub> (conRE), to the direct repeat DNA element AGGTCAAGGTCA (DR0), and to extended half-sites such as TCAAGGTCA (XRE) has been demonstrated, but no natural GCNF target gene has been identified. However, due to an overlapping temporal expression pattern and the presence of DR0-type elements in their promoter regions, the protamine 1 and 2 genes have been proposed as potential candidates for a regulation by GCNF. Since GCNF binds as a homodimer to all three elements (conRE, DR0, and XRE) the receptor exhibits an exceptional property within the nuclear receptor superfamily. Homodimeric binding of GCNF to extended half-sites requires the presence of a novel dimerization motif located in the putative helix 3 of the GCNF ligand-binding domain (LBD). Since neither potential ligands nor heterodimerization partners or cofactors for GCNF have been identified, little is known about the mechanisms by which the receptor controls transcriptional processes. Due to the lack of a conserved transcriptional activation function 2 core motif (AF-2 AD core) in the helix 12 region of the GCNF LBD, it has been suggested that GCNF functions as a repressor of transcription. In addition, recent data suggest that the helix 12 region displays functions distinct from those in other nuclear receptors and is involved in the control of DNA binding. Together, these reports indicate that GCNF exhibits novel properties distinct from other members of the nuclear receptor superfamily.

**Key words** Nuclear orphan receptor · Cell-specific expression · Receptor dimerization · DNA-binding · Transcriptional regulation

**Abbreviations** *AF-2* Activation function 2 · *COUP-TF* Chicken ovalbumin upstream promoter transcription factor · *CTE* C-terminal extension · *DBD* DNA-binding domain · *GCNF* Germ cell nuclear factor · *HR* Hinge region · *LBD* Ligand-binding domain · *NGFI-B* Nerve growth factor inducible receptor B · *NTD* N-terminal domain · *RAR* Retinoic acid receptor · *RXR* Retinoid X receptor · *SF1* Steroidogenic factor 1 · *TR* Thyroid hormone receptor



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## Introduction

Nuclear hormone receptors form a superfamily of ligand-activated transcription factors that play important roles in development, differentiation, and homeostasis [1–5]. They exert their biological effects by binding to *cis*-acting elements, so-called hormone response elements, in the promoter region of target genes. Depending on the particular DNA-binding and dimerization properties, members of the nuclear receptor superfamily bind as homo- or heterodimers to either palindromic or direct repeat response elements [1, 3, 6]. In addition, nuclear orphan receptors have been identified that bind as monomers to extended half-site sequences [6–8].

Nuclear receptors are organized in a modular manner and consist of several functional domains important for DNA binding, homo- and heterodimerization, ligand-binding, transcriptional activation, and repression (Fig. 1) [1]. The structural features of nuclear receptor DNA-binding domains (DBDs) and ligand-binding domains (LBDs) are of central importance for the understanding of receptor function. For a number of nuclear receptors both domains have been characterized in structural as well as in functional detail. The DBD is highly conserved within the nuclear receptor superfamily. It contains two zinc-finger motifs that mediate specific DNA binding via the amino acid residues of the P-box [9–12] which recognize a DNA core sequence (e.g., AGGTCA). Several nuclear receptors including orphan receptors such as steroidogenic factor 1 (SF1) and the nerve growth factor inducible nuclear receptor B (NGFI-B), which can bind DNA as monomers, contact additional base pairs at the 5' end of the core sequence. These protein-DNA contacts are mediated by an amino acid motif, the so-called A-box, located in the C-terminal extension (CTE) of the DBD [7, 8, 13, 14]. In addition to mediating specific DNA binding, the DBD (including the CTE) of most nuclear receptors contains a dimerization function (e.g., the D-box motif) which stabilizes homo- or heterodimeric receptor interactions on DNA [12, 13–17].

The LBDs of nuclear receptors harbor a variety of functions including dimerization, ligand-binding, transcriptional repression, and activation [2, 18]. The elucidation of crystal structures of receptor LBDs significantly contributed to understanding the LBD function [19–22]. The LBD fold (termed  $\alpha$ -helical sandwich) is composed of 12  $\alpha$ -helices (H1–H12) that are packed against one another in a sandwich-like manner [19, 20]. Multiple sequence alignments of nuclear receptor LBDs suggest that this fold is conserved within the superfamily [23]. Comparison of the crystal structures of hormone-bound (holo-) LBDs with unliganded (apo-) LBDs has demonstrated that ligand-binding induces major conformational changes within the  $\alpha$ -helical sandwich structure [19–22]. Most importantly, the helix 12 (H12) of the LBD which harbors the AF-2 AD core [24–26] undergoes a significant structural reorientation and folds back onto the LBD surface [20–22]. The conformational changes are currently believed to destroy interaction surfaces between the receptor and tran-

scriptional corepressors [27, 28] while simultaneously creating novel surfaces for the interaction with transcriptional coactivators [4, 19–22, 29]. Modifications such as phosphorylation [30, 31] and mutations that mimic the effect of ligands [32] can also induce conformational changes within the LBD which generate constitutive active receptors. Thus, the exact conformation of the LBD and the precise orientation of H12 determine the transcriptional properties of a nuclear receptor [22].

In addition to a dimerization function located in the DBD of nuclear receptors, a second dimerization motif is located within the LBD. This motif has been particularly well characterized for the retinoid X receptor (RXR) [19, 33–35]. It is located in the C-terminal part of the LBD and is constituted mainly by helices 9 and 10. These helices are critically involved in forming the dimer interface. Dimerization surfaces similar to that of hRXR $\alpha$  have been proposed to contribute to dimer formation of other nuclear receptors including the retinoic acid receptor (RAR), the thyroid hormone receptor (TR), and the chicken ovalbumin upstream promoter transcription factor (COUP-TF) [35, 36]. Recently a novel dimerization motif comprising the putative helices 5–7 of the LBD of the nuclear orphan receptor “short heterodimer partner” (SHP) has been described which mediates interactions between SHP and RXR, RAR, and TR [37].

Compared to the detailed knowledge about the structure and function of DBDs and LBDs, relatively little is known about the functions of the other domains within a nuclear receptor. The so-called hinge region (HR) which connects the DBD to the LBD is not well conserved within the superfamily and varies considerably in length. The hinge region might be involved in processes such as DNA bending [38] and has been reported to interact with corepressors [27]. The N-terminal domain (NTD) is also not well conserved within the nuclear receptor superfamily. In some cases the NTD harbors a cell type and/or promoter-specific transcriptional activation function (AF-1) [39–41]. The activity of the AF-1 can be regulated by secondary modifications such as phosphorylation [39–41].

In addition to the receptors for retinoic acids, thyroid hormone, vitamin D, and steroid hormones, various related gene products have been cloned for which no ligands have yet been identified. These transcription factors are referred to as nuclear orphan receptors [42, 43]. This review summarizes recent data on the cloning of the nuclear orphan receptor, germ cell nuclear factor (GCNF) from various species and its tissue specific expression pattern. In addition, we compare the DNA-binding and dimerization properties of GCNF with the characteristics of other members of the nuclear receptor superfamily. Taken together, these data show that GCNF displays some unexpected features indicating novel functional and regulatory properties.

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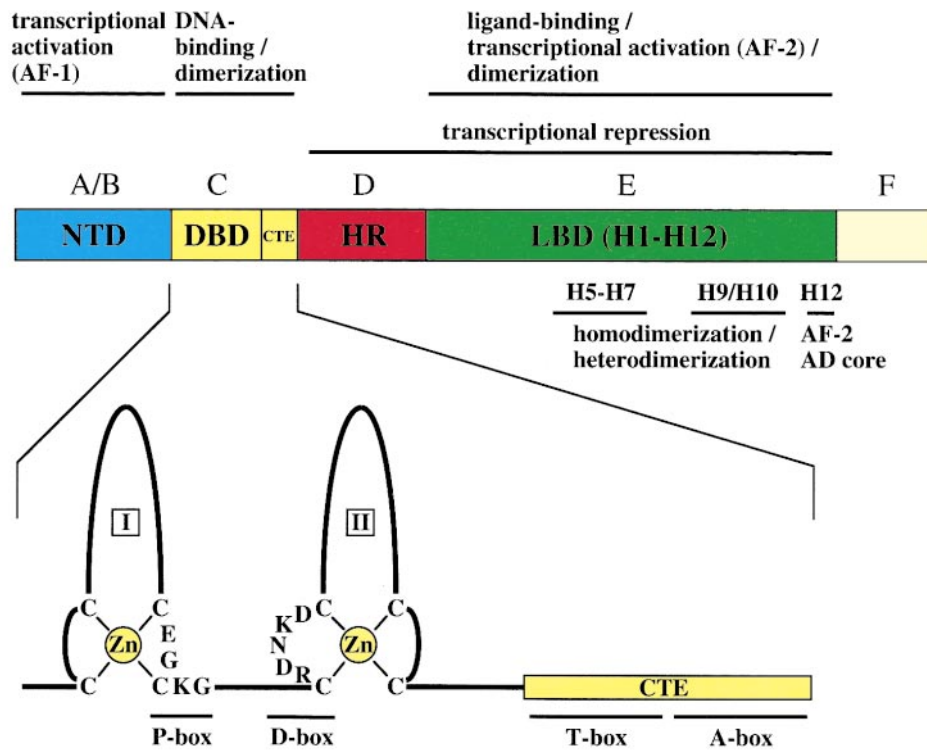
## Cloning and domain organization of GCNF

The nuclear orphan receptor GCNF was initially cloned as a 495 amino acid residue protein from murine tissue via

low stringency cross-hybridization [44] and reverse transcription polymerase chain reaction [45]. Hirose et al. [45] referred to the cloned murine GCNF (mGCNF) as retinoic acid receptor-related testis associated receptor (RTR). Later the cloning of human GCNF (hGCNF) [46–50] and *Xenopus laevis* GCNF (xGCNF) [51] was reported. In addition, Süsens et al. [52] reported the cloning of mGCNF from embryonic tissue, and Bauer et al. [53] cloned mGCNF from a cDNA library of neuronal derivatives of retinoid acid induced embryonal carcinoma cells. Due to a proposed function in the control of neurogenesis Bauer et

al. [53] referred to mGCNF as neuronal cell nuclear factor (NCNF). Recently Southern blot analyses revealed that the (most likely) single hGCNF gene is located on chromosome 9 at the locus q33–34.1 [47, 49], while the mGCNF gene is located on chromosome 2 [49].

With the exception of deletions in the N-terminus, mGCNF, hGCNF, and xGCNF share a high overall homology (Fig. 2). The amino acid identity is about 98% between mGCNF and hGCNF [46–48] and about 84% between the murine and the *X. laevis* receptor [51]. Until now four splice variants of the human receptor have been identified,



	conserved region 1	conserved region 2	conserved region 3	References
mGCNF	1-75 (NTD)	141-160 (DBD)	262-495 (LBD)	[44,45,52,53]
hGCNF-1	1-48 (98%)	56-122 (100%)	141-243 (99%)	[46]
hGCNF-2a	1-51 (92%)	60-126 (100%)	145-247 (99%)	[46,47,48]
hGCNF-2b	1-51 (92%)	60-126 (100%)	145-246 (99%)	[48]
hGCNF-3	1-5 (1-5)	6-28 (34%)	100-119 (99%)	[49]
xGCNF	1-14 (39%)	80-99 (99%)	202-435 (84%)	[51]

all of which have internal deletions in the N-terminal region in comparison with mGCNF [46–49]. One of these hGCNF variants contains in addition a single amino acid deletion ( $\Delta$ Ser-179) in the hinge region [48]. (Alternatively, the absence of Ser-179 might represent an allelic variation.) The N-terminus of xGCNF is 61 amino acids shorter than the corresponding region of mGCNF [51]. To date nothing is known about potential functions of the N-terminus of GCNF. Thus it is unclear whether the different splice variants of the human receptor have distinct functional properties. Figure 2 schematically depicts the GCNF domain organization and lists all identified GCNF proteins.

Sequence alignments of mGCNF with other nuclear receptors reveal the typical domain organization and identify three regions of high conservation (Fig. 2) [44]. The DBD of mGCNF shares the highest amino acid homology (about 60%) with RXR and RAR [44, 45]. The other two conserved regions are located within the LBD of mGCNF and have significant homologies with the corresponding

◀ **Fig. 1** Domain organization of nuclear hormone receptors. Nuclear hormone receptors are composed of several functional domains (A–F) [1]. The NTD may contain a cell type or promoter-specific transcriptional activation function (AF-1) [39–41]. The DBD contains two class II zinc finger motifs which form specific DNA contacts via the amino acid residues of the P-box [9–12]. The P-box motif EGCKG is found in members of the RAR/TR subfamily of nuclear receptors and specifies the binding to an AGGTCA core sequence. In several nuclear receptors a CTE consisting of the T- and the A-boxes is found adjacent to the DBD. Amino acids within the A-box contribute to DNA binding and contact base pairs at the 5' end of the AGGTCA core sequence [7, 8, 13, 14]. In addition to mediating specific DNA binding, the DBD (including the CTE) also contains a dimerization function (e.g., the D-box motif) [12–17]. The HR separates the DBD (including the CTE) from the LBD and has been reported to interact with corepressors [27]. The LBD performs a variety of functions including ligand binding, dimerization, transcriptional activation (AF-2), and repression [18–22]. Elucidation of the LBD crystal structures revealed a folding into 12  $\alpha$ -helices (H1–H12) [19–22]. Ligand-dependent transcriptional activation of nuclear hormone receptors requires the presence of the activation function core motif (AF-2 AD core) located in H12 of the LBD [19–22, 24–26]. Motifs that contribute to homo- or heterodimerization of nuclear receptors have been found in H5–H7 and H9/H10 of the LBDs [19, 33–35, 37]. Some nuclear receptors contain an additional domain (F-domain) adjacent to the LBD. The structure and function of this F-domain are unclear

◀ **Fig. 2** Schematic comparison of mGCNF, hGCNF, and xGCNF. The receptor is composed of the NTD, DBD with an adjacent CTE, HR, and putative LBD. *Horizontal lines*, regions that display significant conservation with other nuclear receptors [44]; *numbers above the schematic representations*, approximate domain borders. Four splice variants of GCNF have been identified in humans which, in comparison with mGCNF, have internal deletions of 19 (hGCNF-1), 15 (hGCNF-2a and hGCNF-2b), or 41 (hGCNF-3) amino acids in the NTD. One variant (hGCNF-2b) carries an additional deletion of serine 179 in the HR. (Alternatively, the absence of serine 179 might represent an allelic variation.) The *X. laevis* receptor contains an additional serine residue (Ser-123, *asterisk*) in the HR which is not present in mGCNF and hGCNF. *Numbers within the schematic drawings*, percentage of identical amino acids in comparison with mGCNF. While the proportion of identical amino acids between xGCNF and mGCNF in the HR and LBD is only 77% and 84%, respectively, the overall homology is much higher due to multiple conserved changes

regions of COUP-TFI, mRXR $\alpha$ , mSF-1, and members of the steroid receptor family [44]. The conservation of amino acid residues in the GCNF LBD that constitute the hydrophobic core in the canonical nuclear receptor LBD fold [23] suggests that the LBD of GCNF adopts an  $\alpha$ -helical sandwich structure (H. Greschik et al., submitted) similar to hRXR $\gamma$ , hRAR $\alpha$ , hTR $\alpha$ , and hER $\alpha$  [19–22]. However, at the very C-terminus differences between the LBDs of GCNF and other nuclear receptors have been observed [48]. Most importantly, an AF-2 AD core in the H12 region of the GCNF LBD is not conserved [48]. Furthermore, secondary structure predictions indicate the presence of a  $\beta$ -sheet and/or coil structure rather than an  $\alpha$ -helical folding of the H12 region (H. Greschik et al., submitted; J.M. Wurtz, D. Moras, personal communication). Since the structure and function of the H12 region are currently unclear, we are left with the possibility that the H12 region serves functions different from transcriptional activation.

Based on phylogenetic studies, Escriva et al. [54] divided the nuclear receptor superfamily into 6 subfamilies and 26 groups of receptors. Interestingly, the subfamily VI is defined by GCNF as the only member identified so far. Since no obvious relationship between the phylogenetic position of a liganded receptor and the chemical nature of its ligand has been observed, the authors suggest that ligand-binding has been acquired independently by the members of the superfamily during evolution [54]. This idea implies that functional properties other than ligand binding may have diverged in the course of evolution. Since GCNF is the only subfamily member identified to date, this receptor may have acquired characteristic features that may not be found in other subfamilies.

## Expression pattern of GCNF

Initial analysis of the expression patterns of mGCNF [44, 45] and hGCNF [46] identified two mRNAs of about 2.3 kb and 7.5 kb that have been detected predominantly in the testis. Significantly lower amounts of the 7.5 kb mRNA are detected in other tissues such as ovary, liver, kidney, lung, and brain [44, 45]. In situ hybridization studies reveal mGCNF expression in the spermatogenic cells of the testis and in the developing oocytes of the ovary [44]. An initial expression analysis in various testicular cell types has indicated that mGCNF mRNA is most abundant in round spermatids [45].

### Expression in germ cells

Recently Katz et al. [55] and Zhang et al. [56] described in detail the temporal and spatial expression pattern of mGCNF in germ cells. In mice mGCNF mRNA is detected during specific stages of spermatogenesis, with the highest mRNA levels in stage VII and VIII round spermatids. Analysis of the temporal expression pattern of the 2.3-kb and 7.5-kb mRNAs indicates that the expression of

these transcripts is differentially regulated [56]. While the 7.5-kb mRNA is detected in spermatocytes and spermatids, the 2.3-kb mRNA seems to be restricted to spermatids [56]. Consistent with mGCNF expression during late stages of spermatogenesis, mGCNF mRNA is not found in the testis of hypogonadal mice [57, 58] that do not develop spermatogenic cells beyond the first prophase of meiosis [55]. While for the murine receptor highest mRNA levels are found in spermatids with weaker signals in spermatocytes, the reverse seems to be the case for hGCNF. The highest hGCNF transcript levels are observed in late spermatocytes with weaker signals in spermatids [49]. The differences in the temporal expression pattern between hGCNF and mGCNF mRNAs have been hypothesized to be due to underlying differences in spermatogenesis in these species [49].

In maturing oocytes of the ovary mGCNF mRNA is found before the first meiotic division [55]. mGCNF expression has been observed only in growing follicles, not in primordial oocytes before the start of follicular growth. Differences in the timing of mGCNF expression in the testis and in oocytes has led to the suggestion that mGCNF is not involved in the regulation of genes that are essential for the basic mechanisms of the meiotic reduction division [55].

Similar to the murine and the human receptor, xGCNF is also expressed in the testis and in oocytes [51]. However, in northern blot analysis lower xGCNF mRNA levels are found in the testis of *X. laevis* than in oocytes. In situ hybridization studies have detected the highest levels of xGCNF mRNA in early diplotene oocytes, but have failed to detect the low amounts of mRNA in the testis [51]. Furthermore, different transcript sizes have been observed for xGCNF than for the human and murine receptors, namely 2.4 kb, 7.5 kb, 8.5 kb, and 10 kb in the oocytes and 8.5 kb in the testis.

It is currently unclear whether the different mRNA sizes in mouse, human, and *X. laevis* reflect the expression of different GCNF isoforms with potentially different functions [49, 51]. Alternatively, the variation in mRNA size may indicate multiple polyadenylation sites or different regulatory elements in the 3' untranslated region, which may lead to altered mRNA stability or differential translation of the transcripts [48, 49, 51, 56]. Most importantly, since the translation of mRNAs during final stages of haploid sperm cell differentiation is known to be strictly controlled [59, 60], it is of major importance to determine when the different GCNF mRNAs are eventually translated into GCNF proteins.

In differentiating sperm cells of the testis mGCNF appears to act as one of the few postmeiotic transcription factors. For comparison, the nuclear orphan receptor TAK1 is expressed at earlier stages of spermatogenesis in diploid spermatocytes [61]. Therefore TAK1 has been suggested to control gene expression during the meiotic phase of spermatogenesis [61]. However, the presence of hGCNF mRNA and the 7.5-kb mRNA of mGCNF in spermatocytes [49, 56] indicates that the receptor may also act during earlier stages of spermatogenesis.

## Expression during development

In addition to the potential regulatory role of GCNF in germ cell development, the receptor may also be involved in the control of neurogenesis and/or neuronal differentiation. Initial evidence came from the analysis of xGCNF expression during embryonic development of *X. laevis* [51]. xGCNF mRNA is first observed in the anterior ectoderm at the end of gastrulation. Zygotic expression of xGCNF peaks at midneurula, where the mRNA was found predominantly in the neural plate and the neural crest. In later embryonic stages (i.e., in the late tailbud stage) xGCNF expression is no longer detectable [51].

Northern blot analysis of mGCNF expression during early stages of murine development [52] has revealed high mRNA levels in 8.5- and 9.5-day-old embryos, decreased transcript levels at day E10.5, and no detectable signal from day E11.5. Compared to the 2.3- and 7.5-kb mRNAs in the murine testis, only the 7.5-kb mRNA is found in embryos. In situ hybridization studies [52] have detected mGCNF mRNA first at about day E6.5 in the embryonic ectoderm, with lower mRNA levels in the extraembryonic ectoderm. During the primitive streak stage (day E7.5) all three germ cell layers as well as the ectoplacental cone exhibit mGCNF expression. At later stages (days E8.5 and E9.5) mGCNF transcripts are observed throughout the neuroectoderm and in the branchial arches, with the highest signal intensity at the marginal edges of the neural plate. After the formation of the neural tube mGCNF mRNA is detected in the ependymal layer and the marginal area. From day E10.5 mGCNF expression decreases with weak signals found in the dorsal root ganglia of the peripheral nervous system and in the marginal layers of the hindbrain and the midbrain. In summary, mGCNF expression is observed during early stages of murine neurogenesis, with the expression pattern becoming restricted to specific zones during later stages of development. Due to the expression of mGCNF in dividing neuroblasts of the ependymal layer and in differentiating neurons of the marginal zone of the neural tube it has been proposed that mGCNF serves two distinct functions in the formation of the nervous system [52].

Another in situ hybridization analysis by Bauer et al. [53] suggests mGCNF expression in adult mice in neuronal cells of all brain regions, including the forebrain, midbrain, and hindbrain. Analyzing the expression of the receptor during embryonic development (starting at day E15) mGCNF mRNA is detected in the neuroepithelium, the trigeminal ganglion and the peripheral nervous system [53]. The observation that mGCNF expression is confined to zones that contain postmitotic cells of the neuronal lineage (e.g., the marginal zone) but is absent in zones that contain mitotic neuronal progenitor cells (e.g., the ventricular germinal zone) led to the proposal that the receptor participates in the stabilization of neuronal lineage determination [53].

In agreement with the potential role of GCNF during early embryonic development, the receptor is expressed in several embryonal carcinoma cell lines and in embryonic stem cells [46, 47, 53, 62]. hGCNF has been cloned from

an embryonal carcinoma cell line (NT2/D1) which expresses markers characteristic of neuroectoderm cells [46]. Upon retinoic acid induced differentiation of NT2/D1 cells hGCNF mRNA has been found to be down-regulated within a few days [47]. In contrast, in the embryonal carcinoma cell line PCC7-Mz1, which differentiates exclusively into neuronal derivatives, mGCNF expression is reported to be induced within hours after retinoic acid treatment [53]. Recently Heinzer et al. [62] described the induction of a DR0-binding protein complex upon retinoic acid exposure of P19 embryonal carcinoma cells. The DNA-binding specificities and immunological properties of this complex suggest that it most likely contains cellular mGCNF [62]. The induction of this DR0-binding complex peaks about 24 h after retinoic acid exposure of the P19 cells and returns to basal levels after about 90 h. Interestingly, the appearance of the cellular mGCNF complex precedes the first markers of neuronal differentiation in P19 cells, and the complex is absent in P19 cells that have undergone neuronal differentiation. Therefore the authors suggested that mGCNF plays a role in cell fate determination rather than in neuronal differentiation [62].

In summary, these reports support the idea that GCNF exerts transcriptional control during gametogenesis and embryonic development. While the temporal expression pattern of the receptor in the ovary suggests a role in premeiotic transcriptional mechanisms, it is currently unclear whether in the testis GCNF acts premeiotically, postmeiotically, or in both ways. Differences in the temporal pattern between hGCNF and mGCNF mRNAs raise the question as to when GCNF proteins are eventually synthesized. Furthermore, while a similar temporal expression pattern of xGCNF and mGCNF indicates a role for the receptor in early embryonic development, questions remain about the expression of GCNF in late neurogenesis and in postnatal brain. Recent results on the expression of GCNF during retinoic acid induced differentiation of embryonal carcinoma cells currently favor the idea that the receptor is involved in cell fate determining processes during early stages of neurogenesis. However, it needs to be clarified whether the temporal pattern of GCNF induction observed in P19 cells resembles the induction pattern in other embryonal carcinoma cells. Finally, it will be interesting to learn whether the reported retinoic acid mediated induction of GCNF expression is a direct effect of nuclear retinoic acid receptors.

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### DNA-binding properties

The first step toward identifying target genes of a nuclear receptor is to characterize its DNA-binding properties. The amino acid sequence similarity between the DBDs of GCNF and RXR, especially in the P-box, suggests binding of GCNF to an AGGTCA core motif [44]. In addition, the amino acid sequence of the A-box displays homology with the corresponding segment of the nuclear orphan receptor SF-1. This suggested that GCNF, as with SF-1, would recognize a TCA extension at the 5' end of the AGGTCA

core [44, 47]. In agreement with the prediction, mGCNF was shown to bind to the extended core motif TCAAGG-TCA (XRE) and to a direct repeat of the core motif AGG-TCAAGGTCA (DR0) [44]. Initial results by Chen et al. [44] suggested homodimeric binding of mGCNF to both the XRE and the DR0. Homodimeric binding to a DR0 has also been reported for hGCNF [63] and xGCNF [51]. In addition, Yan et al. [64] applied an independent electrophoretic mobility shift assay/polymerase chain reaction based binding site selection strategy. They identified TCA[AG(G/T)TCA]<sub>2</sub> as a consensus binding site (conRE) for mGCNF. These authors also showed homodimeric binding of mGCNF to a DR0, but favored monomeric binding to a XRE [64]. In an attempt to clarify the mode of mGCNF binding to a XRE, we demonstrated homodimeric GCNF binding to both XRE and DR0 (H. Greschik, et al., submitted). Recently, homodimeric binding to a XRE was also shown by Cooney et al. [65]. Homodimeric binding to an extended half-site is a specific property of mGCNF, since other orphan receptors, such as SF-1 and NGFI-B bind to extended half-sites as monomers [11, 12]. Deletions within the N-terminus of mGCNF do not significantly affect the DNA-binding properties of the truncated receptor [44] (H. Greschik, et al., submitted). Therefore it seems likely that also hGCNF and xGCNF (which differ from mGCNF mainly in the N-terminus) can bind as homodimers to a XRE. Possible physiological implications of the exceptional DNA-binding mode of GCNF are discussed below after summarizing its dimerization properties.

Despite the relative simplicity of GCNF DNA-binding sites and the detailed knowledge about the spatial and temporal GCNF expression pattern, no target genes have yet been identified. Potential candidates in the developing sperm cell include the transition protein and the protamine genes [66, 67]. Transition proteins and protamines are highly basic sperm-specific nuclear proteins that serve to compact DNA in differentiating sperm cells during late spermiogenesis [66, 67]. Spermatid nuclear condensation starts in elongating spermatids and is thought to be initiated by the transition proteins and completed by the protamines [67]. The transition protein and protamine genes are transcribed at an earlier stage in round spermatids [60] and thus have a temporal expression pattern similar to GCNF [45, 55]. The translation of transition protein and protamine mRNAs needs to be strictly controlled since premature translation of protamine 1 mRNA has been reported to cause precocious nuclear condensation and an arrest of spermatid differentiation which results in infertility in mice [59]. Similarly, defects in the transcription of transition protein and protamine genes may also result in defective spermatid differentiation. Interestingly, the promoters of the protamine 1 and 2 genes have been reported to contain putative GCNF response elements to which the receptor binds *in vitro* [55, 64]. Therefore GCNF may be involved in the transcriptional control of processes that result in spermatid nuclear condensation. However, more evidence is required for a direct involvement of GCNF in the transcription of the protamine 1 and 2 genes.

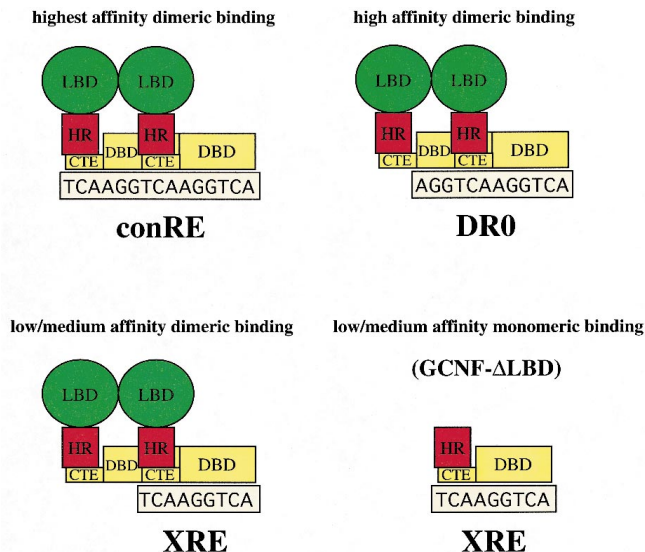
In a recent publication Heinzer et al. [62] report the presence of DR0 binding sites in the promoter region of the proto-oncogene *c-sis* (PDGF-B). Since this gene is temporarily upregulated on days 2 and 3 after treating P19 cell monolayers with retinoic acid [68], it may be a target for regulation by GCNF [62].

### Dimerization properties

Homo- and heterodimerization of nuclear receptors is a common mechanism in the regulation of transcription [6, 69–71]. Dimerization motifs are commonly found in the DBDs (including the adjacent CTE) of nuclear receptors [10, 13–17] and in the C-terminus (H9/H10) of their LBDs [19, 34–36]. In addition, a recently identified novel dimerization motif of the nuclear orphan receptor SHP is located within H5–H7 of its LBD [37]. Dimeric binding of mGCNF to various response elements such as conRE, DR0, and XRE indicates the presence of specific protein-protein interactions that stabilize the DNA-bound homodimer (Fig. 3).

A dimerization motif similar to that in the LBD of RXR has been proposed to be located in the putative H9/H10 of the GCNF LBD [63]. However, the deletion of this putative dimerization motif does not affect homodimeric binding of GCNF to a DR0 [63]. In agreement with these result, our studies reveal that homodimeric binding to a XRE is mediated by a novel dimerization motif located in the putative H3 of the mGCNF LBD (H. Greschik, et al., submitted). Furthermore, point mutations suggest that the H12 region contributes to the dimerization surface in the wild-type receptor. Our data also suggest that the mGCNF LBD can exist in distinct conformations. The conformation of the wild-type mGCNF LBD resembles the holo-LBDs of liganded nuclear hormone receptors. In contrast, the deletion or dislocation of the H12 region induces a conformation similar to unliganded nuclear receptor LBDs. The two different conformations display distinct dimerization properties and do not form heterodimers on DNA. Therefore the H12 region of the mGCNF LBD, which unlike other nuclear receptors lacks a conserved AF-2 AD core, may control a transition between distinct mGCNF dimerization conformations (H. Greschik, et al., submitted).

Deletion of the dimerization motif in H3 of the mGCNF LBD generates a truncated receptor (mGCNF- $\Delta$ LBD) that binds as a monomer to a XRE, suggesting that the dimerization motif in the mGCNF LBD is absolutely required for homodimeric binding to this site (Fig. 3; H. Greschik, et al., submitted). However, mGCNF- $\Delta$ LBD can still bind as a homodimer to a DR0 (H. Greschik, et al., submitted), indicating the presence of a second dimerization motif within the mGCNF DBD. In summary, current data suggest that homodimeric binding of GCNF to a XRE or a DR0 is stabilized by two dimerization motifs. One dimerization motif is located within the putative H3 of the GCNF LBD, and a second may be present within the DBD (including the CTE) of GCNF. Further studies are necessary to characterize the contribution of this second putative



**Fig. 3** Summary of the observed DNA-binding and dimerization properties of GCNF. The binding of GCNF to DNA is mediated by its DBD, which recognizes an AGGTCA core motif, and the CTE, which recognizes a TCA motif 5' of the core sequence. Thus, GCNF binds to the extended core sequence TCAAGGTCA (*XRE*) and the direct repeat element AGGTCAAGGTCA (*DR0*) [44]. An electrophoretic mobility shift assay/polymerase chain reaction based binding site selection strategy identified TCA(AGG/TTCA)<sub>2</sub> as the optimal consensus binding sequence (*conRE*) for GCNF [64]. Homodimeric binding of GCNF to all three sites has been reported [44, 64, 65] (H. Greschik et al., submitted), describing an exceptional property within the nuclear receptor superfamily. The binding of GCNF homodimers to a DR0 and a XRE indicates additional protein-protein interactions that compensate for the loss of specific receptor-DNA contacts with the *conRE*. A novel nuclear receptor dimerization motif has been identified in the putative helix 3 of the GCNF LBD (H. Greschik et al., submitted). The deletion of this dimerization motif generates a truncated receptor (GCNF- $\Delta$ LBD) that binds as a monomer to a XRE (H. Greschik et al., submitted). Since deletions within the N-terminus do not significantly influence the DNA-binding and dimerization properties of GCNF [44] (H. Greschik et al., submitted), the N-terminus has been omitted for reasons of clarity

dimerization motif to homodimeric DNA binding of the receptor.

In XRE-bound GCNF homodimers the individual receptor molecules are apparently nonequivalent in their protein-DNA interactions (Fig. 3). To date nothing is known about possible physiological consequences of this nonequivalence of DNA binding. However, the mode of DNA binding is reminiscent of heterodimeric interactions between members of the nuclear receptor family. For example, in NGFI-B/RXR heterodimers RXR can be tethered to DNA-bound NGFI-B as a cofactor via protein-protein interactions of the receptor LBDs [69]. The allosteric LBD interactions generate a novel heterodimeric complex that is responsive to RXR agonists [69]. Alternatively, transcriptional activation by DNA-bound SF-1 can be inhibited by interactions with the orphan receptor DAX-1 which lacks a conventional DBD [70]. While DAX-1 does not affect DNA binding of SF-1, the orphan receptor SHP (which also lacks a conventional DBD) has been proposed

to block transcriptional activation by tethering RXR into a complex that no longer binds DNA [71]. It is tempting to speculate that GCNF is involved in similar regulatory processes and, for example, is tethered to unidentified XRE-bound heterodimerization partners. However, RXR, the common heterodimerization partner for a number of nuclear receptors, does not interact with GCNF [63], and no evidence for the existence of heterodimerization partners has been found to date. Therefore it has been proposed that GCNF controls transcription independently of dimerization partners [62, 63].

Furthermore, since no transcriptional cofactors have yet been reported, the transcriptional framework in which GCNF exerts its effects remains to be elucidated. Interestingly, the recently identified putative mGCNF complex, which is induced upon retinoic acid exposure of P19 cells, migrates with a lower mobility than in vitro translated mGCNF [62]. An equal complex also forms post-translationally upon mixture of in vitro translated mGCNF with COS-7 cell extracts [62], providing first direct evidence that the receptor interacts with cellular proteins.

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### Transcriptional properties and regulation

Compared to the detailed knowledge of the GCNF expression pattern and the emerging picture of its DNA-binding and dimerization properties, little is known about the way in which the receptor controls transcriptional processes. Some groups have reported significant GCNF-mediated repression from DR0-containing reporter genes in transient transfection assays [53, 65], while other results indicate that GCNF represses transcription only marginally [64] (H. Greschik, P. Hublitz, R. Schüle, unpublished data). In several reports GCNF-mediated repression has seemed to result from overexpression of the receptor to unphysiologically high concentrations. Alternatively, since the transient transfection experiments were performed in various cell lines, the results may indicate cell-type specificity of GCNF-mediated repression. Interestingly, a chimeric receptor in which the GCNF LBD is fused to the heterologous Gal4 DBD acts as a potent repressor of transcription, while the wild-type receptor displays no significant transcriptional activity under the same conditions (H. Greschik, P. Hublitz, R. Schüle, unpublished data).

Although these results suggest that GCNF can act as a repressor, the mechanisms by which GCNF represses transcription remain to be fully elucidated. Potential mechanisms include (a) the active silencing of transcription, (b) the occupation of DNA-binding sites of transcriptional activators, (c) the formation of non-DNA-binding heterodimers with a transcriptional activator, and (d) the inhibition of active receptors via allosteric heterodimeric interactions. For example, transcriptional repression by the nuclear orphan receptor COUP-TF can be mediated by the mechanisms (a) to (c) [72, 73], while the inhibition of DNA-bound SF-1 by DAX-1 exemplifies mechanism (d) [70]. Active silencing by nuclear receptors can be achieved, for example, by nonproductive interactions with

the basal transcription machinery or by recruitment of corepressors [27, 28, 74].

Transcriptional repression by the heterologous Gal4 (DBD)–GCNF (LBD) fusion protein (H. Greschik, P. Hublitz, R. Schüle, unpublished data) is indicative of active silencing. However, the mechanistic details of this process remain to be elucidated. Competition for binding sites has been proposed as another mechanism by which GCNF controls transcriptional processes [51, 53]. Interestingly, during the embryonic development of *X. laevis* xGCNF and xCOUP-TF display a partially overlapping spatiotemporal expression pattern [51]. Since both receptors bind DR0 type elements, they have been proposed to compete for the same target genes [51]. Due to the different DNA-binding affinities of GCNF to the reported binding sites (see Fig. 3) some potential target genes might be more affected by the site competition than others. In addition, the deletion of the H12 region or specific point mutations displacing the H12 region of the mGCNF LBD significantly reduce the binding of mGCNF to a DR0 or a XRE (H. Greschik et al., submitted). These results suggest that in contrast to other nuclear hormone receptors, in which the relative position of H12 determines the transcriptional properties of a given receptor [19–22, 30–32], the H12 region of GCNF may also be involved in the regulation of DNA binding. Upon potential secondary modifications or the binding (or dissociation) of potential ligands, the relative position of the H12 region might be efficiently redirected. Such a mechanism might regulate the ability of GCNF either to bind or to dissociate from a binding site and thus constitute a fine tuning process for the DNA binding of the receptor (H. Greschik et al., submitted).

It is currently unclear whether the lack of a conserved AF-2 AD core in the H12 region of the LBD results in a general incapability of GCNF to activate transcription. The lack of transcriptional activation by GCNF may also be due to the lack of an activating ligand. Several retinoic acid derivatives have been tested as potential ligands but did not affect the transcriptional properties of the receptor [53]. It has thus been suggested that GCNF controls transcription independently of a ligand [53]. However, it is still too early to rule out the existence of a ligand for GCNF. In any case, if the receptor is able to transactivate (either dependent on or independent of a ligand), it will be interesting to learn about a mechanism that compensates for the lack of an AF-2 AD core in the LBD. Possible mechanisms by which GCNF may be activated include secondary modifications such as phosphorylation and allosteric interactions with putative dimerization partners or cofactors.

Since it is possible that GCNF contributes to the regulation of the protamine genes, it is worth speculating by which mechanism(s) the receptor may control the expression of these genes. If GCNF can be activated by a putative ligand or secondary modifications, the receptor would upregulate protamine expression. Alternatively, GCNF could potentially downregulate the expression of the protamine genes during the final phase of spermatid differentiation. However, a first step toward verifying one or the oth-

er hypothesis requires the exact time to be determined when GCNF mRNA is translated into GCNF protein.

In summary, the transcriptional properties and the physiological functions of GCNF are largely unknown. Characterization of the cellular context in which GCNF modulates transcription, identification of target genes, and analysis of GCNF knockout mice (see comment below) should help to contribute to understanding the physiological roles of the receptor. Nevertheless, advances in elucidating the transcriptional framework are a prerequisite for understanding GCNF function. Recent results in the structural and functional characterization of GCNF indicate that this receptor possesses novel properties, and more unexpected features may be unraveled.

### GCNF knockout mice

Data on mGCNF knockout mice were presented by Katz et al. [75]. The mGCNF gene was mutated by homologous recombination with a targeting vector lacking the exon encoding the zinc fingers. Mice heterozygous for the mutant allele appear to behave and reproduce normally, while homozygous null mice die during embryonic development. The proportion of homozygous mutated embryos declines between days 10.5 and 11.5 of embryonic development to almost zero. At this stage the homozygous mutated embryos are significantly delayed in growth and development and display completely open neural tubes. In addition, the critical link between the chorion and the allantois, which is normally made at about day 11, does not form in mGCNF knockout mice. Since mGCNF expression is also observed in the proliferating areas of the ectoplacental cone [52, 75], which are the precursors of placental tissue, the receptor may also play a role in placental development. However, it is currently unclear whether embryonal malformations such as the failure of neural tube closure is a direct effect of the lack of mGCNF expression in this tissue or rather an indirect effect resulting from defects in placental development.

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### References

- Evans RM (1988) The steroid and thyroid hormone receptor superfamily. *Science* 240:889–895
- Gronemeyer H, Laudet V (1995) Transcription factors 3: nuclear receptors. *Protein Profile* 2:1173–1308
- Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schütz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM (1995) The nuclear receptor superfamily: the second decade. *Cell* 83:835–839
- Chambon P (1996) A decade of molecular biology of retinoic acid receptors. *FASEB J* 10:940–954
- Perlmann T, Evans RM (1997) Nuclear receptors in Sicily: all in the famiglia. *Cell* 90:391–397
- Mangelsdorf DJ, Evans RM (1995) The RXR heterodimers and orphan receptors. *Cell* 83:841–850
- Wilson TE, Paulsen RE, Padgett KA, Milbrandt J (1992) Participation of non zinc-finger residues in DNA binding by two nuclear orphan receptors. *Science* 256:107–110
- Wilson TE, Fahrner TJ, Milbrandt J (1993) The orphan receptors NGFI-B and steroidogenic factor 1 establish monomer binding as the third paradigm of nuclear receptor-DNA interaction. *Mol Cell Biol* 13:5794–5804
- Danielsen M, Hinck L, Ringold GM (1989) Two amino acids within the knuckle of the first zinc finger specify DNA response element activation by the glucocorticoid receptor. *Cell* 57:1131–1138
- Umesono K, Evans RM (1989) Determinants of target gene specificity for steroid/thyroid hormone receptors. *Cell* 57:1139–1146
- Mader S, Kumar V, de Verneuil H, Chambon P (1989) Three amino acids of the oestrogen receptor are essential to its ability to distinguish an oestrogen- from a glucocorticoid-responsive element. *Nature* 338:271–274
- Schwabe JWR, Chapman L, Finch JT, Rhodes D (1993) The crystal structure of the estrogen receptor DNA-binding domain bound to DNA: how receptors discriminate between their response elements. *Cell* 75:567–578
- Rastinejad F, Perlmann T, Evans RM, Sigler PB (1995) Structural determinants for nuclear receptor assembly on DNA direct repeats. *Nature* 375:203–211
- Gronemeyer H, Moras D (1995) How to finger DNA. *Nature* 375:190–191
- Lee MS, Kliewer SA, Provencal J, Wright PE, Evans RM (1993) Structure of the retinoid X receptor  $\alpha$  DNA binding domain: a helix required for homodimeric DNA binding. *Science* 260:1117–1121
- Zechel C, Shen XQ, Chambon P, Gronemeyer H (1994) Dimerization interfaces formed between the DNA binding domains determine the cooperative binding of RXR/RAR and RXR/TR heterodimers to DR5 and DR4 elements. *EMBO J* 13:1414–1424
- Zechel C, Shen XQ, Chen JY, Chen ZP, Chambon P, Gronemeyer H (1994) The dimerization interfaces formed between the DNA binding domains of RXR, RAR and TR determine the binding specificity and polarity of the full-length receptors to direct repeats. *EMBO J* 13:1425–1433
- Qi JS, Desai-Yajnik V, Greene ME, Raaka BM, Samuels HH (1995) The ligand-binding domains of the thyroid hormone/retinoid receptor gene subfamily function in vivo to mediate heterodimerization, gene silencing, and transactivation. *Mol Cell Biol* 15:1817–1825
- Bourguet W, Ruff M, Chambon P, Gronemeyer H, Moras D (1995) Crystal structure of the ligand-binding domain of the human nuclear receptor RXR $\alpha$ . *Nature* 375:377–382
- Renaud JP, Rochel N, Ruff M, Vivat V, Chambon P, Gronemeyer H, Moras D (1995) Crystal structure of the RAR- $\gamma$  ligand-binding domain bound to all-*trans* retinoic acid. *Nature* 378:681–689
- Wagner RL, Apriletti JW, McGrath ME, West BL, Baxter JD, Fletterick RJ (1995) A structural role for hormone in the thyroid hormone receptor. *Nature* 378:690–697
- Brzozowski AM, Pike ACW, Dauter Z, Hubbard RE, Bonn T, Engström O, Öhman L, Greene GL, Gustafsson JA, Carlquist M (1997) Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* 389:753–758
- Wurtz JM, Bourguet W, Renaud JP, Vivat V, Chambon P, Moras D, Gronemeyer H (1996) A canonical structure for the ligand-binding domain of nuclear receptors. *Nat Struct Biol* 3:87–94
- Danielian PS, White R, Lees JA, Parker MG (1992) Identification of a conserved region required for hormone dependent transcriptional activation by steroid hormone receptors. *EMBO J* 11:1025–1033
- Barettino D, Vivanco Ruiz MM, Stunnenberg HG (1994) Characterization of the ligand-dependent transactivation domain of thyroid hormone receptor. *EMBO J* 13:3039–3049

26. Durand B, Saunders M, Gaudon C, Roy B, Losson R, Chambon P (1994) Activation function 2 (AF-2) of retinoic acid receptor and 9-cis retinoic acid receptor: presence of a conserved autonomous constitutive activating domain and influence of the nature of the response element on AF-2 activity. *EMBO J* 13: 5370–5382
27. Hörlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Soderstrom M, Glass CK, Rosenfeld MG (1995) Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature* 377:397–404
28. Chen JD, Evans RM (1995) A transcriptional co-repressor that interacts with nuclear hormone receptors. *Nature* 377:454–457
29. Glass CK, Rose DW, Rosenfeld MG (1997) Nuclear receptor coactivators. *Curr Opin Cell Biol* 9:222–232
30. Weis KE, Ekena K, Thomas JA, Lazennec G, Katzenellenbogen BS (1996) Constitutively active human estrogen receptors containing amino acid substitutions for tyrosine 537 in the receptor protein. *Mol Endocrinol* 10:1388–1398
31. White R, Sjöberg M, Kalkhooven E, Parker MG (1997) Ligand independent activation of the oestrogen receptor by mutation of a conserved tyrosine. *EMBO J* 16:1427–1435
32. Vivat V, Zechel C, Wurtz JM, Bourguet W, Kagechika H, Umamiya H, Shudo K, Moras D, Gronemeyer H, Chambon P (1997) A mutation mimicking ligand-induced conformational change yields a constitutive RXR that senses allosteric effects in heterodimers. *EMBO J* 16:5697–5709
33. Zhang XK, Lehmann J, Hoffmann B, Dawson M, Cameron J, Graupner G, Hermann T, Tran P, Pfahl M (1992) Homodimer formation of retinoid X receptor induced by 9-cis retinoic acid. *Nature* 358:587–591
34. Zhang XK, Salbert G, Lee MO, Pfahl M (1994) Mutations that alter ligand-induced switches and dimerization activities in the retinoid X receptor. *Mol Cell Biol* 14:4311–4323
35. Perlmann T, Umesono K, Rangarajan PN, Forman BM, Evans RM (1996) Two distinct dimerization interfaces differentially modulate target gene specificity of nuclear hormone receptors. *Mol Endocrinol* 10:958–966
36. Rosen ED, Beninghof EG, Koenig RJ (1993) Dimerization interfaces of thyroid hormone, retinoic acid, vitamin D, and retinoid X receptor. *J Biol Chem* 268:11534–11541
37. Seol W, Chung M, Moore DD (1997) Novel receptor interaction and repression domains in the orphan receptor SHP. *Mol Cell Biol* 17:7126–7131
38. McBroom LDB, Flock G, Giguère V (1995) The nonconserved hinge region and distinct amino-terminal domains of the ROR $\alpha$  orphan receptor isoforms are required for proper DNA bending and ROR $\alpha$ -DNA interactions. *Mol Cell Biol* 15:796–808
39. Bunone G, Briand PA, Miksicek RJ, Picard D (1996) Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation. *EMBO J* 15: 2174–2183
40. Rochette-Egly C, Adam S, Rossignol M, Egly JM, Chambon P (1997) Stimulation of RAR alpha activation function AF-1 through binding to the general transcription factor TFIID and phosphorylation by CDK7. *Cell* 90:97–107
41. Taneja R, Rochette-Egly C, Plassat JL, Penna L, Gaub MP, Chambon P (1997) Phosphorylation of activation functions AF-1 and AF-2 of RAR alpha and RAR gamma is indispensable for differentiation of F9 cells upon retinoic acid and cAMP treatment. *EMBO J* 16:6452–6465
42. Kastner P, Mark M, Chambon P (1995) Nonsteroid nuclear receptors: what are genetic studies telling us about their role in real life? *Cell* 83:859–869
43. Enmark E, Gustafsson JA (1996) Orphan nuclear receptors – the first eight years. *Mol Endocrinol* 10:1293–1308
44. Chen F, Cooney AJ, Wang Y, Law SW, O'Malley BW (1994) Cloning of a novel orphan receptor (GCNF) expressed during germ cell development. *Mol Endocrinol* 8:1434–1444
45. Hirose T, O'Brien DA, Jetten AM (1995) RTR: a new member of the nuclear receptor superfamily that is highly expressed in murine testis. *Gene* 152:247–251
46. Süsens U, Borgmeyer U (1996) Characterization of the human germ cell nuclear factor gene. *Biochim Biophys Acta* 1309: 179–182
47. Lei W, Hirose T, Zhang LX, Adachi H, Spinella MJ, Dmitrovsky E, Jetten AM (1997) Cloning of the human orphan receptor germ cell nuclear factor/retinoid receptor-related testis-associated receptor and its differential regulation during embryonal carcinoma cell differentiation. *J Mol Endocrinol* 18:167–176
48. Kapelle M, Kratzschmar J, Husemann M, Schleuning WD (1997) cDNA cloning of two closely related forms of human germ cell nuclear factor (GCNF). *Biochim Biophys Acta* 1352: 13–17
49. Agoulnik IY, Cho Y, Niederberger C, Kieback DG, Cooney AJ (1998) Cloning, expression analysis and chromosomal localization of the human nuclear receptor gene GCNF. *FEBS Lett* 424:73–78
50. Schneider-Hirsch S, Bauer UM, Heiermann R, Rentrop M, Maelicke A (1998) Cloning of the human NCNF gene. *J Recept Signal Transduct Res* 18:1–13
51. Joos TO, David R, Dreyer C (1996) xGCNF, a nuclear orphan receptor is expressed during neurulation in *Xenopus laevis*. *Mech Dev* 60:45–57
52. Süsens U, Aguiluz JB, Evans RM, Borgmeyer U (1997) The germ cell nuclear factor mGCNF is expressed in the developing nervous system. *Dev Neurosci* 19:410–420
53. Bauer U-M, Schneider-Hirsch S, Reinhardt S, Pauly T, Maus A, Wang F, Heiermann R, Rentrop M, Maelicke A (1997) Neuronal cell nuclear factor: a nuclear receptor possibly involved in the control of neurogenesis and neuronal differentiation. *Eur J Biochem* 249:826–837
54. Escriva H, Safi R, Hänni C, Langlois MC, Saumitou-Laprade P, Stehelin D, Capron A, Pierce R, Laudet V (1997) Ligand binding was acquired during evolution of nuclear receptors. *Proc Natl Acad Sci USA* 94:6803–6808
55. Katz D, Niederberger C, Slaughter GR, Cooney AJ (1997) Characterization of germ cell-specific expression of the orphan nuclear receptor, germ cell nuclear factor. *Endocrinology* 138: 4364–4372
56. Zhang YL, Akmal KM, Tsuruta JK, Shang Q, Hirose T, Jetten AM, Kim KH, O'Brien DA (1998) Expression of germ cell nuclear factor (GCNF/RTR) during spermatogenesis. *Mol Reprod Dev* 50:93–102
57. Cattanach BM, Iddon CA, Charlton HM, Chiappa SA, Fink G (1977) Gonadotropin-releasing hormone deficiency in a mutant mouse with hypogonadism. *Nature* 269:338–390
58. Mason AJ, Hayflick JS, Zoeller RT, Young III WS, Phillips HS, Nikolics K, Seeburg PH (1986) A deletion truncating the gonadotropin-releasing hormone gene is responsible for hypogonadism in the *hpg* mouse. *Science* 234:1366–1371
59. Lee K, Haugen HS, Clegg CH, Braun RE (1995) Premature translation of protamine 1 mRNA causes precocious nuclear condensation and arrests spermatid differentiation in mice. *Proc Natl Acad Sci USA* 92:12451–12455
60. Kleene KC (1996) Patterns of translational regulation in the mammalian testis. *Mol Reprod Dev* 43:268–281
61. Hirose T, Fujimoto W, Yamaai T, Kim KH, Matsuura H, Jetten AM (1994) TAK1: molecular cloning and characterization of a new member of the nuclear receptor superfamily. *Mol Endocrinol* 8:1667–1680
62. Heinzer C, Süsens U, Schmitz TP, Borgmeyer U (1998) Retinoids induce differential expression and DNA binding of the mouse germ cell nuclear factor in P19 embryonal carcinoma cells. *Biol Chem* 379:349–359
63. Borgmeyer U (1997) Dimeric binding of the mouse germ cell nuclear factor. *Eur J Biochem* 244:120–127
64. Yan ZH, Medvedev A, Hirose T, Gotoh H, Jetten AM (1997) Characterization of the response element and DNA binding properties of the nuclear orphan receptor germ cell nuclear factor/retinoid receptor-related testis-associated receptor. *J Biol Chem* 272:10565–10572
65. Cooney AJ, Hummelke GC, Herman T, Chen F, Jackson KJ (1998) Germ cell nuclear factor is a response element-specific

- repressor of transcription. *Biochem Biophys Res Commun* 245:94–100
66. Wykes SM, Nelson JE, Visscher DW, Djakiew D, Krawetz SA (1995) Coordinate expression of the PRM1, PRM2 and TNP2 multigene locus in human testis. *DNA Cell Biol* 14:155–161
  67. Kistler WS, Henriksen K, Mali P, Parvinen M (1996) Sequential expression of nucleoproteins during rat spermiogenesis. *Exp Cell Res* 225:374–338
  68. Mummery CL, van den Eijnden-van Raaij AJM, Feijen A, Freund E, Hulskotte E, Schoorlemmer J, Kruijer W (1990) Expression of growth factors during the differentiation of embryonic stem cells in monolayers. *Dev Biol* 142:406–413
  69. Forman BM, Umesono K, Chen J, Evans RM (1995) Unique response pathways are established by allosteric interactions among nuclear hormone receptors. *Cell* 81:541–550
  70. Ito M, Yu R, Jameson JL (1997) DAX-1 inhibits SF-1-mediated transactivation via a carboxy-terminal domain that is deleted in adrenal hypoplasia congenita. *Mol Cell Biol* 17:1476–1483
  71. Seol W, Choi HS, Moore DD (1996) An orphan receptor that lacks a DNA binding domain and interacts with other receptors. *Science* 272:1336–1339
  72. Cooney AJ, Tsai SY, O'Malley BW, Tsai MJ (1992) Chicken ovalbumin upstream promoter transcription factor dimers bind to different GGTC A response elements, allowing COUP-TF to repress hormonal induction of the vitamin D<sub>3</sub>, thyroid hormone, and retinoic acid receptors. *Mol Cell Biol* 12:4153–4163
  73. Cooney AJ, Leng X, Tsai SY, O'Malley BW, Tsai MJ (1993) Multiple mechanisms of chicken ovalbumin upstream promoter transcription factor-dependent repression of transactivation by the vitamin D, thyroid hormone, and retinoic acid receptors. *J Biol Chem* 268:4152–4160
  74. Hanna-Rose W, Hansen U (1996) Active repression mechanisms of eucaryotic transcription repressors. *Trends Genet* 12:229–234
  75. Katz D, Pereira FA, Cooney AJ, O'Malley BW (1998) Targeted disruption of the orphan receptor GCNF results in embryonic lethality. Presented at the Keystone Symposium, "The Nuclear Receptor Gene Family," Incline Village, Nevada, 28 March–3 April 1998