MOLECULES IN FOCUS

Retinoid X Receptors

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Retinoids are metabolites of retinol (vitamin A), which act as signalling molecules in embryogenesis and as stimulators of cellular differentiation. The potency of retinoids as differentiating agents has led to their successful use in treating skin diseases and some forms of cancer. The retinoid X receptors (RXRs) are receptors for the vitamin A metabolite all-trans retinoic acid, and they are members of the steroid/thyroid hormone receptor superfamily of DNA binding nuclear hormone receptors. The RXRs are also cofactors required for transcription activated by some other members of the steroid/thyroid hormone receptor superfamily, including the all-trans retinoic acid, thyroid hormone, vitamin D and peroxisome proliferator-activated receptors. The biological importance of the RXRs has been demonstrated in recent genetic studies which have shown that some RXR null mutations in mice have phenotypic effects similar to vitamin A deficiency. © 1997 Elsevier Science Ltd. All rights reserved

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INTRODUCTION

Vitamin A (retinol) is essential to mammals. Deficiency can result in a range of developmental and pathological states, such as heart, lung and eye defects, including blindness (Sporn et al., 1994; Blomhoff, 1994; Morriss-Kay, 1992). Retinol is metabolised into a number of biologically active retinoid compounds (Sporn et al., 1994; Blomhoff, 1994; Morriss-Kay, 1992; Mangelsdorf et al., 1995) including all-trans retinoic acid (RA) and 9-cis RA and all-trans 3,4-didehydro RA. The retinoids are important signalling molecules in embryonic development and in cellular differentiation throughout adult life, for example in epithelial and lymphoid differentiation (Sporn et al., 1994; Blomhoff, 1994; Morriss-Kay, 1992). The significance of retinoid signals is reflected in the teratogenic effects of retinoids in humans and other animals (Morriss-Kay, 1992) and in the successful use of retinoids as treatments for some skin diseases and some forms of cancer (Sporn et al., 1994; Blomhoff, 1994).

Understanding of the mechanism of retinoid signalling was dramatically advanced by the cloning of the retinoic acid receptors (RARs) in the late 1980s (Mangelsdorf et al., 1995). The first RAR was isolated through sequence homology to steroid hormone receptors, identifying the RARs as members of the steroid/thyroid hormone nuclear receptor superfamily of transcription factors. Members of this family consist of proteins with six functional domains A to F (Fig. 1).

The retinoid X receptors (RXRs) were identified in 1990 (Mangelsdorf et al., 1995, 1990; Mangelsdorf and Evans, 1995) as orphan receptors for which there was no known ligand (Mangelsdorf and Evans, 1995). High sequence homology with the RARs (about 60% between amino acid sequences of the human RXRα and RARα DNA binding domains) and also their ability to transactivate gene expression in the presence of all-trans RA, albeit at higher than
physiological concentrations, indicated that RXRs were closely related to the RARs (Mangelsdorf et al., 1990). The ligand for the RXRs was later identified as 9-cis RA (Mangelsdorf et al., 1995; Mangelsdorf and Evans, 1995).

Simultaneous with identification as retinoid receptors, RXRs were also identified as the missing protein cofactor required for efficient transcriptional activation by a subclass of superfamily members, including the RARs, thyroid hormone receptor (T3R), peroxisome proliferator-activated receptor (PPAR) and the vitamin D receptor (VDR) (Mangelsdorf and Evans, 1995; Pfahl, 1993) (Fig. 2).

**RXR STRUCTURE**

There are three separate RXR genes in mammals, RXRs α, β and γ. RXRs are highly conserved between species, and homologues have been found in many animals including the fruit fly *Drosophila* (Mangelsdorf et al., 1990; Mangelsdorf and Evans, 1995). RXR genes encode proteins with relative molecular masses of about 50,000, with the functional domain structure common to other superfamily members (Fig. 1). The functions of the various domains of the RXRs have been investigated by biochemical and molecular biological techniques, and more recently by 3D structural analyses (Mangelsdorf and Evans, 1995; Pfahl, 1993; Lee et al., 1993; Schwabe, 1996). The principal functional domains of the RXRs are the DNA binding C domain and the ligand binding E domain which are separated by a flexible hinge formed by the D domain. The ligand binding domain (Mangelsdorf and Evans, 1995; Schwabe, 1996) consists of two large hydrophobic regions, which form a pocket into which the ligand fits. RXR homodimerisation is also mediated by sequences in the ligand binding domain. On ligand binding, RXRs undergo dramatic conformational changes allowing formation of DNA-binding RXR homodimers. Further experiments are required to shed light on the relationship between formation of homodimers or heterodimers and ligand binding. There is a sequence (the AF-2 domain) located towards the carboxy end of the ligand binding domain which can function in *vitro* as a transactivator of transcription (Schwabe, 1996). It is probable that the activation of this region is also altered on ligand binding.

The DNA binding domain is the region of the protein which is highly conserved between superfamily members (Mangelsdorf et al., 1995) consisting of 66 amino acid residues formed into two cysteine type zinc fingers and a carboxy
terminal extension which is found only in RXRs and RXR-binding receptors (RARs, TRs, VDRs, PPARs, etc.) (Mangelsdorf and Evans, 1995; Lee et al., 1993). The zinc finger region contains two α helices, the first of which fits specifically into the major groove of the DNA helix at the response element (Mangelsdorf and Evans, 1995; Lee et al., 1993). The second helix is thought to be the interface for stabilising heterodimerisation on DNA binding. A third helix formed by the carboxy terminal extension is required for binding of RXR homodimers to DNA response elements (Lee et al., 1993).

The A, B and F domains are not highly conserved between RXRs. Sequences in the A and B domains may modulate the transactivation function of the AF-2 domain and could affect the specificity of RXR transcriptional activation (Mangelsdorf and Evans, 1995; Pfahl, 1993).

**RXR SYNTHESIS**

RXR gene expression has been localised by northern blotting and in situ hybridisation in both embryos and adult tissues (Morriss-Kay, 1992; Mangelsdorf and Evans, 1995; Kastner et al., 1995). In adult mammals RXRβ mRNA is widespread, whilst RXRγ and RXRα expression is more restricted. RXRα mRNA is found in (among other tissues) liver, kidney and skin, and RXRγ in muscle and heart. In embryos, RXRα and β are expressed in a range of tissues, but RXRγ mRNA is more restricted in distribution. RAR genes have been shown to give rise to multiple mRNAs which are differentially spliced in the A and B domains (Pfahl, 1993; Kastner et al., 1995). The different mRNAs have different tissue distributions and encode proteins with different functions (Kastner et al., 1995). Differentially spliced RXR mRNAs, with different tissue distributions have also been reported (Mangelsdorf and Evans, 1995; Pfahl, 1993; Kastner et al., 1995).

**BIOLOGICAL FUNCTION**

RXRs are probably present in cells as monomers in equilibrium with heterodimerised RXRs. The two DNA binding domains in heterodimers are positioned head to tail and bind to response elements which are usually upstream of the transcription start sites of genes (Mangelsdorf and Evans, 1995; Pfahl, 1993; Lee et al., 1993; Schwabe, 1996). Response elements consist of two directly repeated hexamer core sequences separated usually by 1–5 nucleotides. The spacing between the core sequences, sequence differences in the core region and sequence context of the response elements all contribute to the specificity of the ligand response (Mangelsdorf and Evans, 1995; Pfahl, 1993). Heterodimers such as RXR-T,R bind in the unliganded form, and transcription is activated on thyroid hormone binding. Binding of 9-cis RA to heterodimerised RXRs is inhibited by some dimer partners, such as T,R. This is not the case for all receptors, RXR-PPAR heterodimers activate transcription in response to either ligand partner, whilst both partners in RXR-RAR heterodimers respond to 9-cis RA. RXR homodimers form on binding of 9-cis RA and act through different response elements to RXR-RAR dimers (Mangelsdorf and Evans, 1995; Pfahl, 1993).

Interactions also occur between RXRs and other transcription factors (Pfahl, 1993); there are also RXR-like orphan receptors, the COUP transcription factors, which can inhibit response of some genes to retinoids (Mangelsdorf and Evans, 1995; Pfahl, 1993). It is clear that the RXRs are at the centre of a complex web of transcriptional control (Fig. 2).

**RXRs: PHARMACOLOGY AND PATHOLOGY**

The complexities of RXR function have made it difficult to identify direct links between specific disorders and RXRs. Synthetic RXR-specific retinoid compounds may provide a means for dissecting RXR-mediated retinoid activities (Mangelsdorf and Evans, 1995; Pfahl, 1993). Use of transgenic mouse technology to generate RXR mutations is providing some insight into RXR function at the whole organism level (Kastner et al., 1995).

RXRα null mutations have been generated (Kastner et al., 1995). These show some phenotypic similarities to RAR null mutant combinations, and also some similarities to vitamin A deficient embryos. The RXRβ null mutant phenotype (Kastner et al., 1995) indicates effects on PPARβ activation which probably requires RXRβ as a cofactor. The phenotype of RXRγ null mutant mice has not yet been described. Studies of RAR null mutations revealed that compensatory activity of other RARs may occur when one RAR is not produced and genetic crosses between different RAR null mutations have been used to dissect the functions of the RARs. Similar crosses have
been made between RXRα and different RAR null mutants: progeny have a spectrum of vitamin A deficiency-like phenotypes indicating a role for RXRα–RAR interactions in transducing retinoid signals in vivo (Kastner et al., 1995). Use of this type of genetic analysis should enable further dissection of RXR functions and of the complex regulatory interactions between the RXRs and other superfamily members.

REFERENCES


