Abnormal spermatogenesis in RXR β mutant mice

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We have generated mouse lines in which the RXR β gene was disrupted by homologous recombination. Approximately 50% of the RXR β homozygous mutants died before or at birth, but those that survived appeared normal except that the males were sterile, owing to oligo-astheno-teratozoospermia. Failure of spermatid release occurred within the germinal epithelium, and the epididymis contained very few spermatozoa that, in addition, exhibited abnormal acrosomes and tails. There was a progressive accumulation of lipids within the mutant Sertoli cells, which were histochemically characterized as unsaturated triglycerides. In old mutant males, progressive degeneration of the germinal epithelium occurred, ending with the formation of acellular lipid-filled tubules. The selective expression of RXR β in Sertoli cells, together with the timing of appearance of the histological abnormalities, suggests that the primary defect resulting from the mutation resides in these cells.

[**Key Words:** Sertoli cells; lipid metabolism defect; male sterility; seminiferous epithelium degeneration; retinoid X receptor]

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The three retinoid X receptors, RXR α , RXR β , and RXR γ , are members of the vertebrate nuclear receptor superfamily (Leid et al. 1992a,b; Mangelsdorf et al. 1992, 1994; Chambon 1994; Giguère 1994; Glass 1994; Kastner et al. 1994b). In the mouse, RXR α and RXR β transcripts appear to be widely expressed in the embryo and in adult tissues, whereas the distribution of RXR γ transcripts is more restricted (Mangelsdorf et al. 1992; Liu and Linney 1993; Dollé et al. 1994; Nagata et al. 1994). In vitro DNA-binding studies and studies in transfected cells cultured in vitro have provided evidence indicating that RXRs could be involved in several signaling pathways. First, as 9-*cis* retinoic acid (RA) (9C-RA)-dependent transcriptional regulators, RXR homodimers may transduce some of the effects of the active retinoid derivatives of vitamin A. Second, RXRs may also play a role in the retinoid signaling pathway, as heterodimeric partners for the retinoic acid receptors (RAR α , RAR β , and RAR γ) that act as all-*trans* RA (T-RA)- or 9C-RA-dependent transcriptional regulators. Third, RXRs may be involved as heterodimeric partners in additional signaling pathways mediated by other nuclear receptors, which include the thyroid hormone receptors (TRs), the vitamin D3

receptor (VDR), and the peroxisome proliferator-activated receptors (PPARs) (for review, see Mangelsdorf et al. 1992; Liu and Linney 1993; Dollé et al. 1994; Nagata et al. 1994; Desvergne and Wahli 1995). Finally, RXRs may also be the heterodimeric partners of a number of orphan nuclear receptors, including members of the FXR/RLD-1/LXR/UR family (Apfel et al. 1994; Song et al. 1994; Forman et al. 1995a; Seol et al. 1995; Teboul et al. 1995; Willy et al. 1995; note that UR has also been named OR-1 or RIP15), members of the NGFI-B/NURR-1 family (Forman et al. 1995b; Perlmann and Jansson 1995), and the orphan receptor MB67 (Baes et al. 1994).

Whether RXRs engaged in heterodimeric associations act as 9C-RA-dependent transcriptional regulators to synergistically control initiation of transcription or are transcriptionally silent, simply allowing their partners to bind efficiently to the response elements of their cognate target genes, is largely unknown. Several studies performed in vitro and in transfected cultured cells have suggested that the 9C-RA-dependent transcriptional activity of the RXR partner is dependent on both the nature and the ligand occupancy of the heterodimeric partner, as well as on the nature of the bound response element (Durand et al. 1994; Kurokawa et al. 1994; Forman et al. 1995b; Perlmann and Jansson 1995; Willy et al. 1995). Interestingly, Roy et al. (1995) have shown recently that, in P19 and F9 embryonal carcinoma cells, RAR- and RXR-specific ligands have synergistic effects to activate

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endogenous RA target genes and to induce differentiation, which indicates that, at least in some cases of RXR/RAR partnerships, both receptors can be implicated in transcriptional activation of target genes.

In contrast to the wealth of information related to the functions of RXRs *in vitro*, very little is known to date concerning the actual physiological role of RXRs *in vivo*. Notably, it is unknown whether, as a receptor for 9C-RA, RXR transduces some of the multiple effects of retinoids. In this respect, it is noteworthy that most of the abnormalities found in vitamin A-deficient fetuses or adult mice are reproduced in mice bearing mutations in one or several RARs, which demonstrate that RARs are involved in the physiological transduction of the RA signal (Lohnes et al. 1993, 1994; Lufkin et al. 1993; Mendelsohn et al. 1994). The possible function of RXR α has been investigated by examining homozygous null mice generated by targeted disruption of the RXR α gene in embryonic stem (ES) cells (Kastner et al. 1994a; Sucov et al. 1994). These mutants exhibit fetal heart and eye defects similar to those occurring in the fetal vitamin A-deficiency (VAD) syndrome, thus suggesting that RXR α mediates some of the effects of vitamin A. Moreover, these studies have revealed a strong synergy between mutations in RAR and RXR genes for the generation of abnormalities that were either less severe in single RXR or RAR mutants or not present at all. It was also shown that some specific RXR/RAR pairs are much more efficient than others at generating some of the abnormalities seen in the fetal VAD syndrome (Kastner et al. 1994a; P. Kastner, M. Mark, and P. Chambon, unpubl.). Taken together, these data strongly suggest that RAR/RXR heterodimers are the major functional units responsible for transducing the retinoid signal during development.

We now report the generation of RXR β null mutant mice. Approximately 50% of these mutants die in utero or very shortly after birth for unknown reasons. Surprisingly, the remaining null mutants are externally indistinguishable from their wild-type or heterozygous littermates. However, RXR β ^{-/-} males are sterile, owing to abnormal germ cell maturation, leading to oligo-as-theno-teratozoospermia. These sperm abnormalities most probably reflect an indispensable function of RXR β in the Sertoli cells, which in prepubertal mutant males start to accumulate lipid droplets. The possible involvement of PPARs in the generation of the testis abnormalities seen in RXR β null mutants is discussed.

Results

RXR β mutant mice

The RXR β gene was disrupted by homologous recombination in ES cells with a replacement-type vector in which the genomic sequences encoding the DNA-binding domain (3' region of exons 3 and 4) were replaced with a PGK-NEO(A⁺) cassette (Fig. 1a,b). Chimeric males derived from two mutant ES clones (HA67 and

Figure 1. The RXR β mutation. (a) Targeting the RXR β gene. A map of the wild-type RXR β gene is shown at the top. (■) Exons (numbering as in Nagata et al. 1994). Probe A is a NotI–KpnI fragment; probe B is a 500-bp BamHI–HindIII fragment immediately upstream of the deleted region; probe C is the HindIII–EcoRI fragment (corresponding to the deleted region). (Sp) SpeI; (K) KpnI; (N) NotI; (B) BamHI; (E) EcoRI; (Nh) NheI; (X) XhoI; (H) HindIII. (b) Targeted ES cells. Southern blot analysis of SpeI-restricted DNA from wild type (WT) and the HA67 and HA9 ES cell clones, analyzed with the 5' probe A. (c) Southern blot analysis of mutant mice. (Top) The analysis of a litter using BamHI-restricted DNA and probe B. (Bottom) Corresponds to an identical blot hybridized with probe C.

HA9) transmitted the mutation, thus generating two lines of mutant mice. For both lines, crosses between heterozygotes (RXR β ^{+/-}) yielded viable homozygote mutants (RXR β ^{-/-}), which were externally indistinguishable from their wild-type and heterozygote littermates. Homozygote females were fertile, but males were sterile (see below).

As expected, a probe spanning the genomic region encoding the RXR β DNA-binding domain did not detect any hybridizing fragment in homozygotes (probe C in Fig. 1c). Therefore, even though RXR β exons 1, 2, and 5–9 are still present in the genome of homozygotes, a functional DNA-binding RXR β protein cannot be produced in the mutants. RT-PCR analysis of total RNA isolated from several tissues showed that transcripts corresponding to exon 2, as well as transcripts containing exons 5–9, were present at a low level in RXR β ^{-/-} mice when compared with wild type (<10%; data not shown). We cannot therefore exclude that some RXR β peptides

could be present at low levels in mutants. In any event, it is unlikely that the abnormalities observed might result from a dominant effect of such putative peptides, because heterozygous animals never displayed any defect. Thus, the present disruption of the RXR β gene most probably corresponds to a null mutation.

Embryonic and perinatal death of a fraction of RXR β ^{-/-} mutants

When genotyped at 2–3 weeks of age, the numbers of RXR β ^{-/-} mutants derived from intercrosses between RXR β ^{+/-} males and either RXR β ^{+/-} or RXR β ^{-/-} females were significantly lower ($P < 0.005$) than expected from a Mendelian ratio (Table 1). A significant deficit was also observed among 1 day-old newborns and 18.5 dpc (days post-coitum) cesarean-delivered fetuses (Table 1), suggesting that some of the missing RXR β ^{-/-} mutants died in utero or shortly after birth.

It appears therefore that RXR β is important for embryonic development in a fraction of embryos. It is presently unclear when this embryonic death occurs and whether the incomplete penetrance of that phenotype results from heterogeneity in the genetic background of the mutants. That the deficit in RXR β ^{-/-} animals is more pronounced in newborns than in cesarean-delivered 18.5 dpc fetuses (Table 1) suggests that death at or shortly after birth also accounts for some of the missing RXR β ^{-/-} mutants. It is noteworthy, however, that the cesarean-delivered mutants had a normal appearance, breathed normally, and could survive for at least 12 hr when left in isolation. Thus, some of the homozygote mutants may be eliminated through selective maternal cannibalism, similarly to what had been reported previously in the case of RAR γ mutants (Lohnes et al. 1993). Even though a few RXR β ^{-/-} mice died during the first weeks after birth, the majority of RXR β ^{-/-} mutants

that survived through the gestational and perinatal periods had a viability comparable with that of wild-type or heterozygous littermates.

Oligo-astheno-teratozoospermia and abnormal spermiogenesis in RXR β ^{-/-} males

Seven RXR β ^{-/-} males were tested for fertility over a 6-month period. None sired any offspring, even though each of them produced many vaginal plugs. The testes of all RXR β ^{-/-} males were normally descended in the scrotum; the genital ducts (i.e., epididymis, vas deferens) and the accessory glands (i.e., seminal vesicle, prostate and bulbourethral glands) were macroscopically and histologically normal (E in Fig. 2b; results not shown).

In contrast to the epididymides of adult wild-type mice, which on histological sections were packed with sperm (H in Fig. 2a), those of adult RXR β ^{-/-} mutants (2–10 months of age) contained only sparse, elongated or sickle-shaped nuclear profiles characteristic of differentiated spermatozoa (H in Fig. 2b) along with unidentified cellular debris. Moreover, whereas almost all spermatozoa removed from wild-type caudal epididymis became highly mobile when suspended in phosphate-buffered saline (see Bishop 1961), ~95% of the RXR β ^{-/-} caudal epididymal spermatozoa remained immobile under the same conditions. A majority of the RXR β ^{-/-} spermatozoa also exhibited a coiling of the tail (Fig. 2c, arrowheads), an abnormality that was rarely seen in wild-type counterparts. It is noteworthy that this coiling was not seen in testicular spermatids and therefore presumably occurred in the epididymis.

Additional abnormalities were revealed by electron-microscopic examination of RXR β ^{-/-} epididymal spermatozoa. Many acrosomes (~30%) appeared indented (large arrow in Fig. 2d,f,g) and/or partially detached from the nuclear envelope (cf. A, Fig. 2e with f and g). Some spermatozoa lacked an acrosome (not shown). Indented acrosomes were also seen in few wild-type spermatozoa (~7%), but this indentation was never as marked as in mutants. Thus, the high frequency of such defects in mutant spermatozoa strongly suggests that the attachment of the acrosomal membrane to the nucleus (N) is impaired in RXR β ^{-/-} mutants. In addition to these acrosomal defects, local failure of the mitochondria to arrange helicoidally around the axoneme in the middle piece of the mutant spermatozoa tail was observed in ~1–2% of the spermatozoa (open arrow and inset in Fig. 2d). This defect was never seen in wild-type epididymal spermatozoa. Interestingly, these abnormal acrosomes and mitochondrial sheaths were also found in step 16 spermatids within the testis (not shown) and therefore correspond to defects in the process of spermiogenesis (i.e., the maturation of spermatids to spermatozoa within the germinal epithelium).

Taken together, these data indicate that oligo-astheno-teratozoospermia (i.e., severe reduction in the number, reduced mobility, and high percentage of abnormalities of the spermatozoa in the semen) is responsible for the sterility of RXR β ^{-/-} males.

Table 1. Viability of RXR β mutants

	RXR β mutant intercrosses				
	$\text{♀ } +/ - \times \text{♂ } +/ -$			$\text{♀ } -/ - \times \text{♂ } +/ -$	
	+/+	+/-	-/-	+/-	-/-
~3-week-old	132 (1)	288 (2.2)	73 (0.55)	128 (1)	38 (0.3)
1-day-old	15 (1)	33 (2.2)	5 (0.3)	70 (1)	29 (0.4)
18.5 dpc fetuses	76 (1)	129 (1.7)	56 (0.7)	66 (1)	39 (0.6)
Expected Mendelian ratio	1	2	1	1	1

The numbers and genotypes of living mice (3 weeks old or 1 day old) or fetuses (delivered by cesarean section at gestational day 18.5) are given. The values (in parentheses) indicate the ratio with respect to the number of wild type (heterozygote intercrosses) or heterozygotes [homozygote/heterozygote crosses].

Figure 2. Comparison of spermatozoa from the caudal epididymis in wild-type (WT) and RXR β ^{-/-} mutants, as indicated. (a,b) Semithin histological sections, illustrating the paucity of mutant spermatozoa. c corresponds to a smear, showing three spermatozoa displaying coiling of their tails. (d–g) Thin sections illustrating malformations of the acrosome and mitochondrial sheaths in mutant spermatozoa; The inset corresponds to a high magnification of the box in d. (A) Acrosomes; (E) epididymal epithelium; (H) head of spermatozoa; (M) mitochondrial sheath; (N) nuclei of spermatozoa; (X) axoneme. The arrowheads, the large arrows, and the open arrows point to the coiled tails and the defects of the acrosomes and mitochondrial sheaths, respectively. Magnification, 430 $\times$ (a–c), 5000 $\times$ (d–g), and 12,500 $\times$ (inset).

Defective spermiation in the mutant seminiferous tubules

The criteria originally described by Oakberg (1956) and revised by Russell et al. (1990) were used to identify the germ cell types and define the stage of the seminiferous epithelium cycle. In brief, germ cell types were identified primarily by the size and shape of their nuclei, the distribution of chromatin in the nucleus, and the position of the nucleus within the cell. Spermatids were also classified according to the size and shape of the acrosomic system. The latter is strongly periodic acid Schiff (PAS)-positive on sections from Bouin-fixed, paraffin-embedded tissue (A in Fig. 3e,f,i) and is deeply stained by toluidine blue on semithin sections from epon-embedded material (e.g., A in Fig. 3c,d). Round spermatid maturation is divided into eight steps (1–8) reflecting the degree of acrosome development. Elongated spermatid maturation is also divided into eight steps (9–16) on the basis of the shape of the nucleus and acrosome and the extent of head elongation. The stage of a given seminiferous tubule is defined by a specific association of germ cell types. In normal mice, there are 12 stages designated I to XII, each corresponding to one of the first 12 steps of

spermiogenesis. At late stage VII, all the nuclei of the elongated spermatids align at the luminal side of the seminiferous epithelium (Fig. 3c), and the spermatozoa are released into the lumen of the seminiferous tubules at early stage VIII. Late stages of the cycle (i.e., IX–XII; see Fig. 3e) only contain one generation of spermatids (instead of two generations at stages I to early stage VIII).

The diameter of the seminiferous tubules of the six 2-month-old and the six 5- to 6-month-old RXR β ^{-/-} males that were analyzed was normal (Fig. 3, cf. T in a and b). The 12 stages of the cycle of the seminiferous epithelium were readily identifiable (Fig. 3c–f; data not shown), and the proportion of the various germ cell types was apparently normal. However, some of the late spermatids failed to align at the luminal side of tubules at late stage VII (unlabeled arrow in Fig. 3d). In addition, almost all stage IX tubules as well as some stage X tubules contained two generations of elongated spermatids, namely, a normal (step 9 or step 10) and a retained generation (step 16; arrows in Fig. 3f). In stage IX tubules, the heads of the retained spermatids were scattered throughout the seminiferous tubule epithelium, being most abundant at the periphery of the tubules, and were often lying close to the basement membrane (arrows in

Figure 3. Failure of spermatid release and progressive testicular degeneration in $RXR\beta^{-/-}$ males. Sections through the testes of wild-type (WT; *a,c,e*) and $RXR\beta^{-/-}$ males (*b,d,f,g-i*) at 6 months (*a-f*), 8 months (*g,i*), and 12 months (*h,j*). (A) Acrosomes; (B) basement membrane; (L) lipid droplets; (P) pachytene spermatocytes; (R) preleptotene spermatocytes; (S) Sertoli cell nucleus; (T) seminiferous tubules; (V) vacuoles of degeneration; (Y) Leydig cells. The numbers in the white dots refer to the degree of maturation (steps) of the spermatids. (Arrowheads in *b,f*) Lipid-containing vacuoles; (unlabeled arrows) retained step 16 spermatids. Note that the focal hyperplasia of the Leydig cells (Y) in *g* and the edema of the intertubular space in *h* represent classical secondary alterations to seminiferous tubule atrophy. Hematoxylin–trichrome (*a,b,g,h*), alcian blue (*c,d*), and PAS–hematoxylin (*e,f,i,j*). Magnification, *a,b,g*, and *h* (111 $\times$); *c-f,i*, and *j* (370 $\times$).

Fig. 3*f*). At the electron-microscopic level, these remnants of elongated spermatids were located within the cytoplasm of Sertoli cells (large arrow in Fig. 4). In addition, these phagocytosed spermatids reacted strongly for the detection of fragmented DNA by the TUNEL method (Gavrieli et al. 1992; data not shown). Therefore, defective spermiation (i.e., spermatid release), accompanied by phagocytosis of the retained late spermatids by the Sertoli cells, occurs in $RXR\beta^{-/-}$ testes. This defect

is likely to account for the near absence of spermatozoa in the epididymides of the $RXR\beta^{-/-}$ mice.

Lipid accumulation in mutant Sertoli cells

Paraffin sections of all adult (i.e., 2 months old and older) $RXR\beta^{-/-}$ testes revealed also the presence of large, rounded, clear “vacuoles” located at the periphery of every seminiferous tubule (arrowheads in Fig. 3, cf. *b* and *f*

Figure 4. Basal portion of a 6-month-old RXR $\beta^{-/-}$ mutant Sertoli cell in a stage IX seminiferous tubule. (L) Lipid droplet; (LI) lysosomes; (S) Sertoli cell nucleus; (arrow) phagocytized elongated spermatid. Magnification, 5400 $\times$.

with a and e). These vacuoles were strongly osmiophilic on semithin sections from resin-embedded material (L in Fig. 3d and Fig. 6d,f,g, below), suggesting that they contained lipids. In unfixed frozen sections, they reacted with osmium tetroxide (Fig. 5, cf. T in c and d), thus demonstrating the unsaturated character of the lipids (see Gabe 1968). The lipid dye oil red O, which stains mainly triglycerides and sterols, also stained strongly the tubules of RXR $\beta^{-/-}$ mutants (Fig. 5, cf. T in a and b), whereas the Schultz's test for sterols gave a negative response (not shown), suggesting therefore that the droplets contain triglycerides. These droplets were also devoid of phospholipids, because they failed to react with MC22-33F, a monoclonal antibody directed against phosphatidylcholine [Fig 5e; note that the lipids present in granulosa cells, the female equivalent of the Sertoli cells, react strongly with this antibody (G in Fig. 5f)]. Together, these histochemical and immunohistochemical properties of the vacuoles indicate that they essentially correspond to droplets of unsaturated triglycerides. Acid phosphatase positive granules (probably lysosomal

Figure 5. Lipids and lysosomal structures in RXR $\beta^{-/-}$ mutant testis. (a–e, g–i) Frozen sections through the testes of 6-month-old wild-type (WT; a,c,g) and RXR $\beta^{-/-}$ (b,d,e,h,i) males. (a,b) Staining with oil red O. (c,d) Staining with osmium tetroxide. (e) Immunofluorescence staining of a RXR $\beta^{-/-}$ seminiferous tubule with the anti-phospholipid antibody (MC22-33F; top) or nonimmune rat IgM (0 control; bottom), on consecutive sections. (f) Immunostaining of an antral follicle with MC22-33F (top) or nonimmune rat IgM (0 control; bottom) on consecutive sections. (g–i) Detection of acid phosphatase activity (after staining with oil red O in i). (B) Level of the basement membrane of the seminiferous tubule; (G) granulosa cells; (L) lipid droplets in Sertoli cells; (N) nuclei; (T) seminiferous tubules; (Y) Leydig cells. Arrows point to lysosomes in i. Magnification, 62 $\times$ (a–d,g,h), 96 $\times$ (e,f), and 412 $\times$ (i).

in nature) were consistently associated with the lipid droplets (arrows in Fig. 5i; cf. T in Fig. 5, g and h). It is noteworthy that in wild-type testis both the triglycerides and the acid phosphatase activity were almost exclusively confined to the Leydig cells (Y in Fig. 5g; data not shown). At the electron-microscopic level, these $RXR\beta^{-/-}$ tubular triglyceride droplets were strictly confined to the cytoplasm of the Sertoli cells and were not limited by a membrane (L in Fig. 4).

To investigate the evolution of the lipid droplets with age, we examined semithin sections of osmium-fixed testis from 29-day-old animals as well as from 2-, 6-, 8-, and 12-month-old animals (Fig. 6a–h). Lipid droplets were scarce and small in wild-type testes at all ages (see, e.g., L in Fig. 3c). In $RXR\beta^{-/-}$ testes, abundant small lipid droplets were already detected in all 29-day-old mutant males analyzed (four animals; Fig. 6, cf. T in a and b). Note that these animals had not yet completed their first round of spermatogenesis (Russel et al. 1990). The size of these lipid droplets increased gradually with the age of the mutant (L in Fig. 6d, f, g), and in 6-month-old

males, some of these droplets were already larger than the Sertoli cell nuclei (L in Figs. 3d, 4, and 6f). In old animals, many tubules were devoid of Sertoli cells but filled with lipids (L in Fig. 6h). It appears therefore that the ultimate death of the Sertoli cells is preceded by a progressive increase in lipid content with age.

It has been suggested that the enzyme "hormone-sensitive lipase" (HSL) may be important for controlling lipid metabolism in Sertoli cells, because it is inactivated in cryptorchid testes, in which lipids accumulate within the Sertoli cells (Stenson-Holst et al. 1994). However, Northern blot analysis of HSL transcript levels in 2 month-old males did not reveal any difference between wild-type and $RXR\beta^{-/-}$ adult testes (data not shown).

Degeneration of the seminiferous epithelium in old $RXR\beta$ mutant mice

Histological sections through both testes of all 8- to 10-month-old $RXR\beta^{-/-}$ mice (five males) showed seminiferous tubules with abnormalities similar to those seen in

Figure 6. Lipid accumulation in $RXR\beta^{-/-}$ mutant testis: Early appearance, prior to the completion of spermatogenesis (29 days), and lipid droplet size increase with the age of the animal, as indicated. Photomicrographs of semithin sections from osmium-fixed testes without (a,b) or with counterstaining (c–h) with alcian blue. Lipids (L) appear as brown or black dots or spots in the seminiferous tubules (T). (Y) Leydig cells; (V) vacuoles of degeneration. Magnification 66 $\times$.

5- to 6-month-old males and, in addition, tubules with reduced diameters that exhibited different degrees of germ cell loss (T in Fig. 3g). These ranged from tubules with normal populations of mitotic (i.e., spermatogonia) and meiotic (e.g., zygotene or pachytene spermatocytes) germ cells, but lacking spermatids, to tubules containing Sertoli cells only. Germ cell loss appeared to result mainly from the sloughing off of apparently healthy immature germ cells (i.e., pachytene spermatocytes and/or round spermatids) into the lumen of the tubules (e.g., step 7 spermatids in Fig. 3i) and was apparently preceded by the appearance of large irregular pockets of empty space in the seminiferous epithelium (V in Fig. 3i). This second type of vacuole, which does not contain lipids (V in Fig. 6g) constitutes in rodent testes an unspecific, early degenerative event (for review, see Yuan and McEntee 1987).

In all testes of 11- to 12-month-old RXR $\beta^{-/-}$ males, up to half of the seminiferous tubules were replaced by tubular ghosts consisting of a thickened and convoluted basement membrane, which were filled with lipids (Figs. 3h,j and 6h).

RXR α and RXR β transcripts' localization and immunolocalization of RXR β in the seminiferous epithelium

The localization of RXR β transcripts in the seminiferous epithelium of wild-type males was analyzed by in situ hybridization. RXR β transcripts were present in every seminiferous tubule (T in Fig. 7b); the strongest signals were detected in the basal portion of the epithelium (where most of the Sertoli cell cytoplasm is located), whereas weaker signals were observed in the more luminal portions (Fig. 7d). Interestingly, the silver grains were often radially aligned in this latter part of the epithelium, which is consistent with a localization in the cytoplasmic processes of Sertoli cells (Fig. 7d; data not shown). Immunoperoxidase staining of wild-type seminiferous tubules with an antibody directed against RXR β showed a positive signal only in the cell nuclei that on the basis of their localization at the periphery of the seminiferous tubules and their triangular shapes were identified as those of the Sertoli cells. Germ cells [e.g., pachytene spermatocytes (P), round spermatids (RS), and elongated spermatids (ES)] were not stained (Fig. 7f). As expected, no staining was detected in the RXR $\beta^{-/-}$ seminiferous epithelium (Fig. 7g). These data indicate that RXR β testicular expression is restricted to Sertoli cells and does not depend on the stage of the seminiferous tubule.

RXR α transcripts were also present in the testis; however, their localization was apparently restricted to some round spermatids in a subset of tubules (Fig. 7c,e). Therefore, within the seminiferous epithelium, the distributions of RXR α and RXR β transcripts do not appear to overlap. In contrast, RXR γ transcripts could not be detected in the testis (data not shown).

Discussion

Abnormal spermatogenesis and degeneration of the seminiferous epithelium

We demonstrate here that RXR β is essential for spermatogenesis in the mouse: RXR β null mutant males exhibit abnormalities in spermiogenesis (i.e., spermatid maturation) and spermiation (i.e., spermatid release). Interestingly, mutant Sertoli cells exhibit concomitantly an early and a progressive cytoplasmic accumulation of unsaturated triglycerides. These observations raise the question whether Sertoli cells and germ cells are independently affected by the lack of RXR β or whether one cell type is primarily affected. Abnormal Sertoli cells could be impaired in their ability to correctly assist the maturation and release of spermatids (Redenbach and Vogel 1991; Jegou 1993; Griswold 1995); alternatively, the lipid accumulation in Sertoli cells could result from widespread phagocytosis of abnormal spermatids (Russell et al. 1990).

Three lines of evidence strongly suggest that Sertoli cells may be primarily affected in RXR β null mutants. First, in wild-type testes, RXR β appears to be solely expressed in Sertoli cells. Second, at puberty, Sertoli cell abnormalities precede by at least a week the appearance of abnormal spermatids in RXR $\beta^{-/-}$ mutants. Lipid droplets were detected in Sertoli cells of 29-day-old mutants, before the completion of the first spermatogenic cycle (Russell et al. 1990 and refs. therein). Therefore, at least the earliest lipid accumulation observed in Sertoli cells cannot be ascribed to the phagocytosis of retained late spermatids. Third, lipid droplets in Sertoli cells were not observed at any stages in testes of RXR α mutant mice (Lufkin et al. 1993; Fig. 8a; data not shown) nor in VAD rats (Huang and Marshall 1983) or mice (Fig. 8b; data not shown), even though there is also in these cases a failure of spermatid release accompanied by a phagocytosis of retained spermatids. Thus, lipid accumulation is not a necessary consequence of impaired spermiation. Taken together, these observations suggest that the accumulation of lipids observed in Sertoli cells of RXR β null mutants reflects a primary metabolic defect.

It is well established that Sertoli cell/germ cell interactions are critical for sperm maturation and spermiation (for review, see Jegou 1993; Griswold 1995). Each maturing spermatid is encased by Sertoli cell cytoplasm that is thought to assist its maturation and its release by as yet poorly understood mechanisms. Thus, the defects of spermiogenesis and spermiation in RXR $\beta^{-/-}$ mutant testes could reflect an altered function of Sertoli cells. The occurrence of large lipid droplets within Sertoli cells has been described in several pathological conditions associated with defective spermatogenesis, such as cryptorchidism (Fleege et al. 1968; for review, see Johnson 1970) or androgen insensitivity (the *tfm* mice; Chung and Hamilton 1975). Furthermore, high levels of lipids are frequently observed in the testes in cases of human sterility (Johnson 1970 and refs. therein). These observations, together with those made here for RXR β null mu-

Figure 7. Comparison of the localization of RXR β (b,d) and RXR α (c,e) transcripts in a wild-type adult testis and immunoperoxidase staining with an antibody directed against RXR β of wild-type (WT; f) and RXR $\beta^{-/-}$ (g) testis. (a) Bright field; (b,c) dark fields. In d and e, the in situ hybridization signal is shown in false colors after computer processing of a bright-field view and dark-field view of the same section (see Vonesch et al. 1994 for further details). In f and g, a strong positive signal is exclusively detected in wild-type Sertoli cells. Immunostaining is absent from wild-type germ cells and from mutant Sertoli cells and germ cells. The weak staining of the mutant and wild-type Leydig cells (L) corresponds to background staining because it can be observed even when omitting the anti-RXR β antibody in the immunostaining sequence. (EP) Epididymis; (T) seminiferous tubules; (S) Sertoli cells or spermatogonia; (P) pachytene spermatocytes; (RS), round spermatids; (ES) elongated spermatids. Magnification, 6 $\times$ (a–c); 95 $\times$ (d–g).

tants, suggest that a faulty lipid metabolism in Sertoli cells could lead to defective spermatogenesis.

The physiological role of lipids during spermatogenesis is unknown. Lipid catabolism might be required solely to generate the energy needed during the transformation of spermatids into spermatozoa and/or their release into the lumen of the seminiferous tubule. Alternatively, some lipids may perform more specific functions as signaling molecules and/or cofactors. In this respect, it is noteworthy that PERF15, the major component of the perinuclear theca (the structure involved in anchoring the acrosomal membrane to the nuclear envelope), has been characterized recently as a homolog of fatty acid-binding protein (FABP) and adipocyte lipid-binding protein (Okamoto and Morales 1994). If a lipid ligand is indeed required for the function of this protein, the unavailability of such a ligand in RXR β null mutants could contribute to the detachment of the acrosomal membrane from the nuclear envelope seen in the spermatozoa of these mutants.

Which signaling pathway is affected in RXR $\beta^{-/-}$ mutant testes?

The defects observed in RXR $\beta^{-/-}$ testes could reflect a

requirement of 9C-RA for spermatogenesis and/or the participation of RXR β as a partner for another nuclear receptor that requires heterodimerization with RXRs to bind to the response elements of its cognate target genes. The roles of retinoids in the testis have been documented from studies of VAD animals (for review, see Eskild and Hansson 1994). VAD testes are characterized by a rapid degeneration of the seminiferous epithelium, because almost all the germ cells are lost within the first 8 days following the initial weight loss (Eskild and Hansson 1994; see also Fig. 8b). It is therefore noteworthy that the RXR $\beta^{-/-}$ seminiferous tubules keep their normal cellular architecture at least until 6 months of age. Thus, the function of retinoids in the maintenance of the structure of the seminiferous epithelium does not appear to be precociously impaired in RXR $\beta^{-/-}$ mutants. Furthermore, extensive lipid accumulation has not been reported in the Sertoli cells of VAD animals (see also Fig. 8b), suggesting that the defect in lipid metabolism seen in RXR $\beta^{-/-}$ mice does not reflect the existence of a 9C-RA-dependent step in this metabolism. The testicular phenotype of RXR α null mutants matches closely the lesions resulting from VAD (Lufkin et al. 1993; Fig. 8) and is clearly different from the RXR $\beta^{-/-}$ mutant phenotype, because testicular degeneration can occur at

Figure 8. Comparison of semithin sections from osmium-fixed, resin-embedded testes of the seminiferous tubules of 6-month-old RAR $\alpha^{-/-}$ mutant (a) and VAD (b) mice. The VAD males were the F1 offsprings of dams fed a VAD diet and raised on a VAD diet from the time of weaning [S. Ward, in prep.]. The large arrows point to similar tubules having lost their germ cells and thus containing only Sertoli cells (S); the cytoplasm of the Sertoli cells contains large vacuoles (V) that are devoid of lipids. (Y) Leydig cells. Magnification, 260 $\times$.

much earlier stages in RAR α mutants (i.e., before the completion of puberty) in the absence of lipid droplet accumulation (P. Gorry, M. Mark, and P. Chambon, unpubl.). Thus, the retinoid signal that is necessary for spermatogenesis appears to be transduced by RAR α , which is expressed in Sertoli cells and spermatids (Eskild et al. 1991), and, if one assumes that RAR α requires a RXR partner for its function in seminiferous epithelium, this partner is most probably RXR α . Whether the function of RXR β in the testis involves in any way the transduction of a retinoid signal will ultimately require the generation of mouse lines bearing mutations that selectively affect the 9C-RA-dependent function(s) of RXR β .

Members of the PPAR superfamily have been implicated recently as transcriptional regulators of several genes involved in lipid metabolism and have been shown to be activated by various fatty acids (for review, see Desvergne and Wahli 1995). The accumulation of lipids in RXR $\beta^{-/-}$ mutant Sertoli cells could therefore reflect an impaired PPAR function. RXR β may act as a heterodimeric partner for PPAR β , which appears to be highly expressed in Sertoli cells (Braissant et al. 1996). Whether RXR β could act as a 9C-RA-independent heterodimeric PPAR partner in the testis will require a ge-

netic analysis of PPAR function in the testis and the generation of compound RXR β /PPARs mutants. In this respect, we note that PPAR α mutant mice have been produced recently, that do not exhibit testicular defects (Lee et al. 1995).

Because RXR β is the most abundant (if not the only) RXR expressed in Sertoli cells, all nuclear receptors that require RXR as a dimerization partner might be functionally impaired in these cells. The present alteration of the lipid metabolism may reflect the impairment of just one of these pathways (possibly the PPAR pathway), and other biochemical defects not detectable at the cytological level may exist in RXR β null mutant Sertoli cells. Thus, the lipid metabolism defect may not necessarily be the primary cause (or the only cause) responsible for the impaired spermatogenesis. In this respect, we note that in the frog *Rana nigromaculata*, a steroid hormone, 17 α ,20 α -dihydroxy-4-pregnen-3-one, induces spermiation (Kobayashi et al. 1993). If a similar molecule is required for mammalian spermiation and if its effect is transduced by a RXR partner, then the lack of RXR β could lead to the defect in spermiation observed in RXR $\beta^{-/-}$ mutants.

Functional redundancy among RXRs

The defect observed in RXR β null mice is surprisingly mild in view of the pleiotropic role that has been postulated for RXRs, either as heterodimeric partners for several nuclear receptors and/or as receptors for 9C-RA (see introductory section for references). Because RXR α and RXR β appear to be widely expressed during embryogenesis and in adult tissues, it is possible that RXR α may functionally compensate for the loss of RXR β in most instances. In this respect, the complete penetrance, as well as the absence of variation in its expressivity, of the lipid metabolism defect observed in Sertoli cells of RXR β null mutants, may correspond to a case in which such a redundancy cannot operate, given the apparent absence of RXR α expression in Sertoli cells. On the other hand, the partial penetrance of the embryonic lethal phenotype of RXR β mutants could result from a partial functional redundancy amongst RXRs. Interestingly, the developmental phenotype of RXR α null mutants affects also a limited set of structures (the heart and the eye; see Kastner et al. 1994a; Sucov et al. 1994). Moreover, the heart malformations seen in RXR $\alpha^{-/-}$ embryos vary greatly in their severity from mutant to mutant, and RXR α /RXR β double null mutants display much more severe developmental abnormalities than RXR $\alpha^{-/-}$ single mutants (P. Kastner and P. Chambon, unpubl.). In particular, their early cardiac defects are much more severe than those presented by RXR α single mutants. Thus, as observed previously for the RARs (Lohnes et al. 1994; Mendelsohn et al. 1994), the RXRs can be functionally redundant, because RXR β appears to be able to substitute for the lack of RXR α in many developmental instances.

Conclusion

The RXR β mutant mice described here offer a new model for further understanding spermatogenesis. In particular, they should provide valuable tools to study the role of Sertoli cells in germ cell maturation and spermiation. Because lipid accumulation has also been observed in the Sertoli cells of human patients with fertility problems, our results raise the possibility that RXR β mutations may be involved in some cases of human male sterility. Finally, these mice, together with mice mutated in other RXRs or putative RXR heterodimeric partners, will be useful to study genetically the physiological role of RXRs and of their interaction with other members of the nuclear receptor superfamily.

Materials and methods

Targeting vectors and homologous recombination

Genomic clones for the mouse RXR β (mRXR β) locus were obtained by screening a genomic library established in λ EMBL3 from 129/Sv mouse DNA with an mRXR β cDNA probe. The structure of the mRXR β locus was identical to that reported by Nagata et al. (1994). To construct the targeting vector, a 10-kb *NotI*–*XhoI* fragment containing the entire RXR β locus was inserted into Bluescript (SK–). A *HindIII* site was then created by site-directed mutagenesis in exon 3 at the boundary between the B and C regions. Subsequently, the 1.5-kb *HindIII*–*EcoRI* fragment containing sequences encoding the entire RXR β DNA-binding domain was removed and replaced with a PGK–NEO(A⁺) cassette (derived from pKJ-1; Adra et al. 1987), which was cloned in antisense orientation. This plasmid, pHR(RXR β) was linearized with *XhoI* and electroporated into D3 ES cells as described previously (Lufkin et al. 1991). After selection with G418, resistant clones were expanded and genomic DNA was prepared, restricted with *SpeI*, and analyzed by Southern blotting with probe A (Fig. 1a). Three positive clones were obtained that were confirmed with *HindIII* or *KpnI* digests using probe A, as well as a NEO probe. All three clones were injected into C57BL/6 blastocysts, and chimeras derived from two clones (HA9 and HA67) transmitted the mutant allele through their germ line. Routine genotyping of the mice was subsequently performed on *BamHI*-restricted DNA using probe B (Fig. 1a,b).

Histological procedures

Sections (7 μ m thick) from Bouin-fixed, paraffin-embedded testes were dewaxed and stained either with Groat's hematoxylin and Mallory's trichrome (Mark et al. 1993) or by the PAS reaction [the Schiff's reagent was made from pararosaniline (C.I. no. 42500; Sigma) according to Longley's method (Gabe 1968)] followed by a 3-min counterstain with Harris hematoxylin solution (Merck).

Histochemistry, immunohistochemistry, and in situ hybridization on frozen tissues

Sections (10 μ m thick) from unfixed frozen tissues were collected on poly-L-lysine-coated slides. The sections were (1) hybridized for 3 min in PBS (prior to staining with oil red O or with osmium tetroxide), (2) fixed for 10 min in 4% paraformaldehyde in PBS at 24°C (prior to the demonstration of acid phosphatase activity), (3) fixed for 3 min in 100% acetone at –20°C, then air-dried (prior to anti-phospholipid immunostaining), or (4)

fixed in Zamboni's fixative for 15 min, then treated with 1% H₂O₂ to block endogenous peroxidase (prior to staining with the anti-RXR β antibody). Staining with the lipid-soluble dye oil red O (C.I. no. 26125; Sigma) in isopropanol/water for 3 min was performed as indicated by Lillie (1965); the sections were counterstained with Harris hematoxylin (5 sec), rinsed in tap water (3 min), then mounted in glycerol–PBS (9:1). Oxidation of unsaturated lipids by osmium tetroxide was achieved by covering the sections with a drop of this fixative (1% in PBS) for 5 min; after rinsing in distilled water, the sections were counterstained with 1% methyl green (C.I. no. 42590; Sigma; purified by chloroform extraction) and mounted in glycerol–PBS. The detection of cholesterol was performed according to Hershberger's modification of the Schultz method (Lillie 1965). The demonstration of acid phosphatase activity in situ was performed according to Burstone, as reported by Lillie (1965): paraformaldehyde-fixed tissue sections were incubated for 2 hr at 24°C in 0.1 M sodium acetate buffer (pH 5.2) containing the substrate naphthol AS-BI phosphate (Sigma) and the diazonium salt fast red violet LB (Sigma). A monoclonal antibody against phosphatidylcholine (MC22-33F; Mark et al. 1992) was used to immunolocalize phospholipid-containing cytoplasmic inclusions on acetone-fixed tissue sections, as described previously. Sections from an adult mouse ovary and the replacement of MC22-33F by nonimmune rat IgM served as positive and negative controls of the immunostaining procedure. Immunostaining with an anti-RXR β antibody (Sugawara et al. 1995; 1/4000 diluted in TBST) was performed using the ABC Elite system (Vector, Burlingame, CA) according to the manufacturer's instructions. Sections of RXR^{–/–} testis reacted with the anti-RXR β antibody served as negative controls.

The RXR probes used for in situ hybridization were synthesized from cDNA fragments covering the entire open reading frame (Dollé et al. 1994). In situ hybridization was performed on 10- μ m frozen sections as described (Décimo et al. 1996), and the sections were stained with hematoxylin.

Electron microscopy

Mice were perfused intracardially with a 2.5% solution of glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Testes and epididymides were removed and immersed in the same fixative overnight at 4°C. They were then rinsed with this buffer, post-fixed for 1 hr at 4°C in cacodylate-buffered osmium tetroxide, dehydrated with graded alcohols, and embedded in epon. For light microscopy, semithin sections (1 μ m thick) were stained with toluidine blue. Ultra-thin sections were contrasted with uranyl acetate and lead citrate and examined with a Philips 208 electron microscope at 80 kV.

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References

- Adra, C.N., P.H. Boer, and N.W. McBurney. 1987. Cloning and expression of the mouse pgk-1 gene and the nucleotide sequence of its promoter. *Gene* **60**: 65–74.
- Apfel, R., D. Benbrook, E. Lernhardt, M.A. Ortiz, G. Salbert, and M. Pfahl. 1994. A novel orphan receptor specific for a subset of thyroid hormone-responsive elements and its interaction with the retinoid/thyroid hormone receptor subfamily. *Mol. Cell. Biol.* **14**: 7025–7035.
- Baes, M., T. Gulick, H.S. Choi, M.G. Martinoli, D. Simha, and D.D. Moore. 1994. A new orphan member of the nuclear hormone receptor superfamily that interacts with a subset of retinoic acid response elements. *Mol. Cell. Biol.* **14**: 1544–1552.
- Bishop, D.W. 1961. Biology of spermatozoa. In *Sex and internal secretions* (ed. W.C. Young), Vol. 2, pp. 707–797. Williams & Wilkins, Baltimore, MD.
- Braissant, O., F. Foulle, C. Scotto, M. Dauça, and W. Wahli. 1996. Differential expression of peroxisome proliferator-activated receptors: Tissue distribution of PPAR α , β , and γ in the adult rat. *Endocrinology* (in press).
- Chambon, P. 1994. The retinoid signaling pathway: Molecular and genetic analyses. *Semin. Cell Biol.* **5**: 115–125.
- Chung, K.W. and J.B. Hamilton. 1975. Testicular lipids in mice with testicular feminization. *Cell Tiss. Res.* **160**: 69–80.
- Décimo, D., E. Georges-Labouesse, and P. Dollé. 1996. In situ hybridization of nucleic acid probes to cellular RNA. In *Gene probes, a practical approach book*. Vol. II. (ed. B.D. Hames and S. Higgins). Oxford University Press, Oxford, UK. (in press.)
- Desvergne, B. and W. Wahli. 1995. PPAR: A key nuclear factor in nutrient/gene interactions? In *Inducible gene expression* (ed. P.A. Baeuerle), Vol. 1, Chap. 5, pp. 142–176. Birkhauser, Boston, MA.
- Dollé, P., V. Fraulob, P. Kastner, and P. Chambon. 1994. Developmental expression of murine retinoid X receptor (RXR) genes. *Mech. Dev.* **45**: 91–104.
- Durand, B., M. Saunders, C. Gaudon, B. Roy, R. Losson, and P. Chambon. 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.
- Eskild, W. and V. Hansson. 1994. Vitamin A functions in the reproductive organs. In *Vitamin A in health and disease* (ed. R. Blomhoff), pp. 531–559. Marcel Dekker, New York, NY.
- Eskild, W., A.H. Ree, F.O. Levy, T. Jahnsen, and V. Hansson. 1991. Cellular localization of mRNAs for retinoic acid receptor- α , cellular retinol-binding protein, and cellular retinoic acid-binding protein in rat testis: Evidence for germ cell-specific mRNAs. *Biol. Reprod.* **44**: 53–61.
- Fleeger, J.L., J.P. Bishop, W.R. Gomes, and N.L. VanDemark. 1968. Testicular lipids. I. Effect of unilateral cryptorchidism on lipid classes. *J. Reprod. Fert.* **15**: 1–7.
- Forman, B.M., E. Goode, J. Chen, A. Oro, D. Bradley, T. Perlmann, D. Noonan, L.T. Kurka, T. McMorris, W. Lamph, R. Evans, and C. Weinberger. 1995a. Identification of a nuclear receptor that is activated by farnesol metabolites. *Cell* **81**: 687–693.
- Forman, B.M., K. Umehono, J. Chen, and R. Evans. 1995b. Unique response pathways are established by allosteric interactions among nuclear receptors. *Cell* **81**: 541–550.
- Gabe, M. 1968. *Techniques histologiques*. Masson et Cie, Paris, France.
- Gavrieli, Y., Y. Sherman, and S.A. Ben-Sasson. 1992. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *J. Cell Biol.* **119**: 493–501.
- Giguère, V. 1994. Retinoic acid receptors and cellular retinoid binding proteins: Complex interplay in retinoid signaling. *Endocrine Rev.* **15**: 61–79.
- Glass, C.K. 1994. Differential recognition of target genes by nuclear receptor monomers, dimers, and heterodimers. *Endocrine Rev.* **15**: 391–407.
- Griswold, M.D. 1995. Interactions between germ cells and Sertoli cells in the testis. *Biol. Reprod.* **52**: 211–216.
- Huang, H.F.S. and G.R. Marshall. 1983. Failure of spermatid release under various vitamin A states—and indication of delayed spermiogenesis. *Biol. Reprod.* **28**: 1163–1172.
- Jegou, B. 1993. The Sertoli-germ cell communication network in mammals. *Int. Rev. Cyt.* **147**: 25–95.
- Johnson, A.D. 1970. Testicular lipids. In *The testis* (ed. A.D. Johnson, W.R. Gomes, and N.L. Vandemark) Vol. 2, pp. 194–258. Academic Press, New York, NY.
- Kastner, P., J.M. Grondona, M. Mark, A. Gansmuller, M. LeMeur, D. Decimo, J.L. Vonesh, P. Dollé, and P. Chambon. 1994a. Genetic analysis of RXR α developmental function: Convergence of RXR and RAR signaling pathways in heart and eye morphogenesis. *Cell* **78**: 987–1003.
- Kastner, P., M. Leid, and P. Chambon. 1994b. Role of nuclear retinoic acid receptors in the regulation of gene expression. In *Vitamin A in health and disease* (ed. R. Blomhoff), pp. 189–238. Marcel Dekker, New York, NY.
- Kobayashi, T., S. Sakai, S. Adachi, K. Asahina, H. Iwasawa, and Y. Nagahama. 1993. 17 α , 20 α -dihydroxy-4-pregnen-3-one is the naturally occurring spermiogenesis-inducing hormone in the testis of a frog, *Rana nigromaculata*. *Endocrinology* **133**: 321–327.
- Kurokawa, R., J. Drenzo, M. Boehm, J. Sugarman, B. Gloss, M.G. Rosenfeld, R.A. Heyman, and C.K. Glass. 1994. Regulation of retinoid signaling by receptor polarity and allosteric control of ligand binding. *Nature* **371**: 528–531.
- Lee, S.T., T. Pineau, J. Drago, E.J. Lee, J.W. Owens, D. Kroetz, P.M. Fernandez-Salguero, H. Westphal, and F.J. Gonzalez. 1995. Targeted disruption of the α isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol. Cell. Biol.* **15**: 3012–3022.
- Leid, M., P. Kastner, R. Lyons, H. Nakshatri, M. Saunders, T. Zacharewski, J.Y. Chen, A. Staub, J.M. Garnier, S. Mader, and P. Chambon. 1992a. Purification, cloning and RXR identity of the Hela cell factor with which RAR and TR heterodimerizes to bind target sequences efficiently. *Cell* **68**: 377–395.
- Leid, M., P. Kastner, and P. Chambon. 1992b. Multiplicity generates diversity in the retinoic acid signalling pathways. *Trends Biochem. Sci.* **17**: 427–433.
- Lillie, R.D. 1965. *Histopathologic technic and practical histochemistry*, 3rd ed. McGraw Hill Book Company, New York, NY.
- Liu, Q. and E. Linney. 1993. The mouse retinoid X receptor- γ

- gene: Genomic organisation and evidence for functional isoforms. *Mol. Endocrinol.* **7**: 651–658.
- Lohnes, D., P. Kastner, A. Dierich, M. Mark, M. LeMeur, and P. Chambon. 1993. Function of retinoic acid receptor γ in the mouse. *Cell* **73**: 643–658.
- Lohnes, D., M. Mark, C. Mendelsohn, P. Dollé, A. Dierich, P. Gorro, A. Gansmuller, and P. Chambon. 1994. Function of the retinoic acid receptors (RARs) during development. I. Craniofacial and skeletal abnormalities in RAR double mutants. *Development* **120**: 2723–2748.
- Lufkin, T., A. Dierich, M. LeMeur, M. Mark, and P. Chambon. 1991. Disruption of the Hox 1.6 homeobox gene results in defects in a region corresponding to its rostral domain of expression. *Cell* **66**: 1105–1119.
- Lufkin, T., D. Lohnes, M. Mark, A. Dierich, P. Gorro, M.P. Gaub, M. LeMeur, and P. Chambon. 1993. High postnatal lethality and testis degeneration in retinoic acid receptor alpha mutant mice. *Proc. Natl. Acad. Sci.* **90**: 7225–7229.
- Mangelsdorf, D., U. Borgmeyer, R. Heyman, J.Y. Zhou, E. Ong, A. Oro, A. Kakizuka, and R. Evans. 1992. Characterization of three RXR genes that mediate the action of 9-cis retinoic acid. *Genes & Dev.* **6**: 329–344.
- Mangelsdorf, D.J., K. Umesono, and R.M. Evans. 1994. The retinoid receptors. In *The retinoids*, 2nd ed. (ed. M.B. Sporn, A.B. Roberts, and D.S. Goodman) pp. 319–338. Raven Press, New York, NY.
- Mark, M.P., T. Tsuiji, J. Portoukalian, A. Rebbaa, G. Zidan, and J.V. Ruch. 1992. Characterization of a monoclonal antibody that specifically binds to choline phospholipids and its use in immunocytochemistry. *J. Histochem. Cytochem.* **40**: 827–838.
- Mark, M., T. Lufkin, J.L. Vonesch, E. Ruberte, J.C. Olivo, P. Dollé, P. Gorro, A. Lumsden, and P. Chambon. 1993. Two rhombomeres are altered in *Hoxa-1* mutant mice. *Development* **119**: 319–338.
- Mendelsohn, C., D. Lohnes, D. Décimo, T. Lufkin, M. LeMeur, P. Chambon, and M. Mark. 1994. Function of the retinoic acid receptors (RARs) during development. II. Multiple abnormalities at various stages of organogenesis in RAR double mutants. *Development* **120**: 2749–2771.
- Nagata, T., Y. Kanno, K. Ozato, and M. Taketo. 1994. The mouse RXR β gene: Genomic organization and two mRNA isoforms generated by alternative splicing of transcripts initiated from CpG island promoters. *Gene* **142**: 183–189.
- Oakberg, E.F. 1956. Duration of spermatogenesis in the mouse and timing of stages of the cycle of the seminiferous epithelium. *Am. J. Anat.* **99**: 507–516.
- Oko, R. and C.R. Morales. 1994. A novel testicular protein, with sequence similarities to a family of lipid binding proteins, is a major component of the rat sperm perinuclear theca. *Dev. Biol.* **166**: 235–245.
- Perlmann, T. and L. Jansson. 1995. A novel pathway for vitamin A signaling mediated by RXR heterodimerization with NGFI-B and NURR1. *Genes & Dev.* **9**: 769–782.
- Redenbach, D.M. and A.W. Vogel. 1991. Microtubule polarity in Sertoli cells: A model for microtubule-based spermatid transport. *Eur. J. Cell Biol.* **54**: 277–290.
- Roy, B., R. Taneja, and P. Chambon. 1995. Synergistic activation of expression of retinoic acid (RA) responsive genes and induction of P19 and F9 EC cell differentiation by a RAR α , a RAR β , or a RAR γ selective ligand in combination with a RXR-specific ligand. *Mol. Cell. Biol.* **15**: 6481–6487.
- Russell, L.D., A.P.S. Hikim, R.A. Ettlin, and E.D. Clegg. 1990. *Histological and histopathological evaluation of the testis*. Cache River Press, Clearwater, FL.
- Seol, W., H.S. Choi, and D.D. Moore. 1995. Isolation of proteins that interact specifically with the retinoid X receptor: Two novel orphan receptors. *Mol. Endocrinol.* **9**: 72–85.
- Song, C., J.M. Kokontis, R.A. Hiipakka, and S. Liao. 1994. Ubiquitous receptor: A receptor that modulates gene activation by retinoic acid and thyroid receptors. *Proc. Natl. Acad. Sci.* **91**: 10809–10813.
- Stenson-Holst, L., A.M. Hoffmann, H. Mulder, F. Sundler, C. Holm, A. Bergh, and G. Frederikson. 1994. Localization of hormone-sensitive lipase to rat Sertoli cells and its expression in developing and degenerating testes. *FEBS Lett.* **355**: 125–130.
- Sucov, H.M., E. Dyson, C.L. Gumeringer, J. Price, K.R. Chien, and R.M. Evans. 1994. RXR α mutant mice establish a genetic basis for vitamin A signaling in heart morphogenesis. *Genes & Dev.* **8**: 1007–1018.
- Sugawara, A., P.M. Yen, Y. Qi, R.M. Lechan, and W.W. Chin. 1995. Isoform-specific retinoid-X receptor (RXR) antibodies detect differential expression of RXR proteins in the pituitary gland. *Endocrinology* **4**: 1766–1774.
- Teboul, M., E. Enmark, Q. Li, A.C. Wikstrom, M. Peltto-Huikko, and J.A. Gustafsson. 1995. OR-1, a member of the nuclear receptor superfamily that interacts with the 9-cis-retinoic acid receptor. *Proc. Natl. Acad. Sci.* **92**: 2096–2100.
- Vonesch, J.L., H. Nakshatri, M. Philippe, P. Chambon, and P. Dollé. 1994. Stage and tissue-specific expression of the alcohol dehydrogenase 1 (Adh-1) gene during mouse development. *Dev. Dynam.* **199**: 199–213.
- Willy, P.J., K. Umesono, E.S. Ong, R.M. Evans, R.A. Heyman, and D.J. Mangelsdorf. 1995. LXR, a nuclear receptor that defines a distinct retinoid response pathway. *Genes & Dev.* **9**: 1033–1045.
- Yuan, Y.D. and K. McEntee. 1987. Testicular degeneration, rat. In *Monographs on pathology of laboratory animals. Genital system* (ed. T.C. Jones, U. Mohr, and R.D. Hunt), pp. 212–225. Springer-Verlag, Heidelberg, Germany.

Abnormal spermatogenesis in RXR beta mutant mice.

P Kastner, M Mark, M Leid, et al.

Genes Dev. 1996, **10**:

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References

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