Nephrol Dial Transplant (2002) 17 [Suppl 9]: 78–80

Nephrology
Dialysis
Transplantation

Retinoids and the Kidney

Vitamin A and kidney development

Thierry Gilbert

INSERM U423, Hôpital Necker—Enfant Malades, Tour lavoisier, 6 ème étage, 149 rue de Sèvres, 75743 Paris Cedex 15, France

Abstract
Recent advances in developmental nephrology have provided new evidence that retinoids, vitamin A and its active metabolites such as all-*trans* retinoic acid, profoundly influence renal organogenesis. Retinoids are acknowledged as potent nephron mass regulators. They regulate embryonic kidney patterning through control of Ret expression in a dose-dependent manner, which modulates ureteric bud branching morphogenesis. Analysis of retinoid availability and utilization provides additional evidence of retinoid involvement at sites of epithelial–mesenchymal interactions, suggesting a tight control of vitamin A homeostasis for proper renal morphogenesis and differentiation. This provides a rationale for the search for and identification of retinoid target genes.

Keywords: c-ret; nephron mass; renal organogenesis; retinoids

During embryogenesis, the kidney develops when the undifferentiated metanephric blastema is invaded by the ureteric bud, the epithelial outgrowth of the Wolffian duct. Inductive interactions between the growing ureteric bud and the metanephric mesenchyme lead to (i) branching morphogenesis of the former to become the collecting duct system; and (ii) conversion of the latter into an optimal number of nephrons [1]. Both processes rely on complex cross-talk between the metanephrogenic mesenchyme and the ureteric bud tips to shape the metanephros architecture. Although the induction-dependent conversion of the mesenchyme into nephrons has been characterized extensively [2], the signals that trigger and control ureteric bud branching morphogenesis remained unclear. Numerous growth factors have been proposed to be involved in ureteric bud branching morphogenesis, e.g. bone morphogenetic proteins (BMPs), glial cell line-derived neurotrophic factor (GDNF) and fibroblast growth factors (FGFs). However, none of them is able on its own to turn on the branching programme. Until recently, the role of vitamin A, a well-characterized signalling molecule involved in differentiation of the whole embryo and cell identity determination [3], was not acknowledged in the developing kidney. Today we know that retinoids, vitamin A or retinol and its active metabolites such as all-*trans* retinoic acid (RA), profoundly influence renal organogenesis *via* expression of specific genes (for a review, see [4]). Some molecular RA targets have been identified, which bring a rationale for the various renal anomalies observed in the offspring of severely vitamin A-deficient dams [5] and in double mutant mice deficient for retinoic acid nuclear receptor isoforms [6]. This brief report sums up our current understanding of the role of vitamin A during kidney development.

The first insights into the molecular mechanisms of retinoids during renal organogenesis came from investigations aimed at identifying RA target genes. Attention was focused initially on components of the GDNF–GDNFRα–Ret signalling complex that is required for renal organogenesis [7]. We took advantage of a model of metanephros organ culture combined with quantitative approaches of nephrogenesis to demonstrate that retinoids are potent stimulators of nephron formation in a dose-dependent manner [8]. When E14 rat metanephroi were grown in serum-free medium, c-*ret* expression dropped significantly within 48 h of culture [9]. The sole addition of exogenous RA in the culture medium maintained c-*ret* expression. Furthermore, when the RA supplementation was performed after 2 days of culture in RA-free medium, c-*ret* expression could be turned on. This is illustrated on Figure 1. Both sets of data were confirmed at the protein level. Then, using semi-quantitative reverse transcription–polymerase chain reacton (RT–PCR), we showed that there was a linear correlation between c-*ret* expression and RA concentration in the culture medium over a broad range of RA concentrations, from 10^{-9} to 10^{-6} M [9]. This relationship suggests that the amount of RA available in the developing kidney is a key factor to ensure optimal c-*ret*

Correspondence and offprint requests to: Thierry Gilbert, PhD, INSERM 423, Hôpital Necker—Enfant Malades, Tour lavoisier, 6 ème étage, 149 rue de Sèvres, 75743 Paris Cedex 15, France.

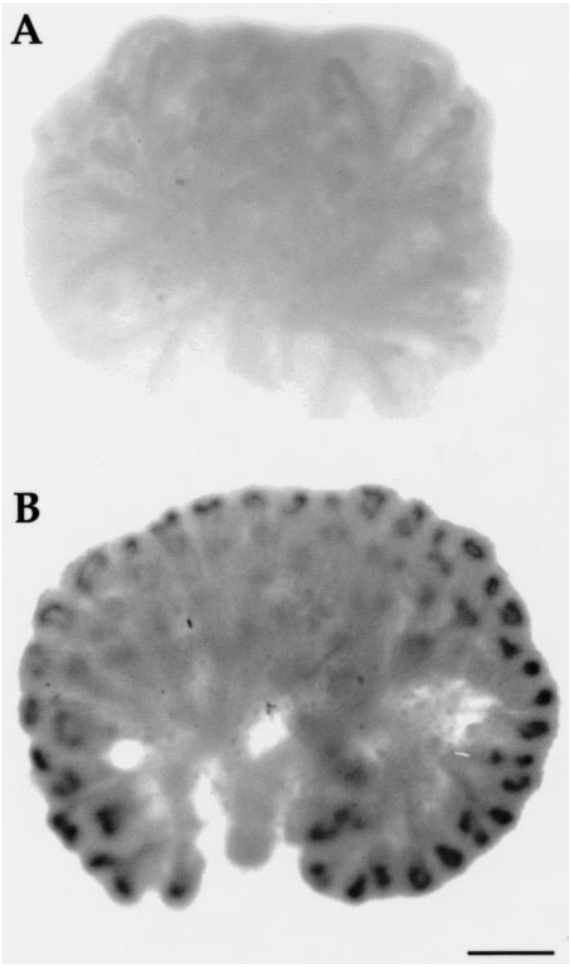

Fig. 1. Whole-mount *in situ* hybridization of c-*ret* expresssion in one pair of E14 rat metanephroi grown in serum-free medium for 4 days without (**A**) or with (**B**) 100 nM of exogenously added RA. The plasmid carrying c-*ret* cDNA was a gift from F. Costantini. The antisense digoxigenin-labelled riboprobe was generated following plasmid linearization with *Bam*HI and synthesis with T3 RNA polymerase. Alkaline phosphatase-conjugated Fab anti-digoxigenin antibody and the NBT–BCIP substrate reaction were used to visualize the hybrids. The presence of RA in the culture medium maintained strong c-*ret* expression at the tips of the ureteric bud.

expression. In addition, c-*ret* expression levels paralleled the number of nephrons. On the contrary, no effect was observed on GDNF or GDNFRα mRNA levels, which remained high throughout *in vitro* metanephros development. In a model of mild vitamin A deficiency, we demonstrated that a 50% reduction in circulating vitamin A in pregnant rats, that did not affect overall fetal development, did induce a reduction of the number of nephrons in term fetuses [10]. Interestingly, the number of nephrons was closely correlated with fetal circulating vitamin A levels. Thus vitamin A supply to the fetus is critical in determining the number of nephrons. Prior to these studies, only large variations in vitamin A status have been considered as risk factors for the fetus. We demonstrated that due to its tight control by vitamin A, renal organogenesis is altered by mild variations in maternal vitamin A status. In mice lacking both RA nuclear receptors, RARα and RARβ2, that displayed renal hypoplasia, c-*ret* expression was down-regulated, leading to impaired branching morphogenesis of the ureteric bud [11]. Altogether, this confirms the pivotal role of the duo vitamin A/c-*ret* in kidney formation.

The second type of information came from characterization of the vitamin A metabolic pathways. Using a reporter cell line, F9-RARE-*lacZ*, we showed that the embryonic kidney was able to release morphogenetically active retinoids to induce β-galactosidase activity. The same result was obtained with microdissected E13 rat ureteric bud and metanephrogenic mesenchyme put on top of these cells, indicating that both tissues contained endogenous retinoids. Since retinol and retinaldehyde were able to promote *in vitro* nephrogenesis, we examined the distribution of enzymes involved in RA synthesis within the developing rat kidney. We focused our attention on two retinaldehyde dehydrogenases, Raldh1 and 2, enzymes that catalyse the limiting step of retinol oxidation. Raldh1 is expressed in the ureteric bud up to its tips and is excluded from the mesenchyme. On the other hand, Raldh2 is excluded from the ureteric epithelium but is highly expressed in the peripheral metanephric mesenchyme. Therefore, two enzymes with a complementary pattern of expression are present in the rat metanephros [12]. The role of this peculiar distribution remains to be clarified. Nevertheless, it suggests that the local availability of active retinoids at the epithelium–mesenchyme interface between the ureteric bud and the metanephrogenic mesenchyme might control nephrogenesis *via* expression of specific genes.

In conclusion, we have demonstrated that the nephron number is closely modulated by the vitamin A environment and we proposed that the fetal vitamin A status may be responsible for most of the variations in nephron numbers found in the general population. Clinical and epidemiological studies on the consequences of fetal vitamin A deficiency should therefore be conducted, as it has been proposed that non-syndromic defects in renal development may have important clinical relevance [13]. In the meantime, we pursue identification of the molecular cascade of vitamin A control of nephrogenesis by searching for additionnal RA target genes using a differential display expression screen.

References

1. Saxén L. *Organogenesis of the Kidney*. Cambridge University Press, Cambridge, 1987
2. Ekblom P. Developmentally regulated conversion of mesenchyme to epithelium. *FASEB J* 1989; 3: 2141–2150
3. Means AL, Gudas LJ. The roles of retinoids in vertebrate development. *Annu Rev Biochem* 1995; 64: 201–233
4. Gilbert T, Merlet-Benichou C. Retinoids and nephron mass control. *Pediatr Nephrol* 2000; 14: 1137–1144

5. Wilson JG, Warkany J. Malformations in the genito-urinary tract induced by maternal vitamin A deficiency in the rat. *Am J Anat* 1948; 83: 357–407
6. Mendelsohn C, Lohnes D, Décimo D *et al*. Function of the retinoic acid receptors (RARs) during development. (II) Multiple abnormalities at various stages of organogenesis in RAR double mutants. *Development* 1994; 120: 2749–2771
7. Sariola H, Saarma M. GDNF and its receptors in the regulation of the ureteric branching. *Int J Dev Biol* 1999; 43: 413–418
8. Vilar J, Gilbert T, Moreau E, Merlet-Bénichou C. Metanephros organogenesis is highly stimulated by vitamin A derivatives in organ culture. *Kidney Int* 1996; 49: 1478–1487
9. Moreau E, Vilar J, Lelièvre-Pégorier M, Merlet-Bénichou C, Gilbert T. Regulation of c-ret expression by retinoic acid in rat metanephros: implication in nephron mass control. *Am J Physiol* 1998; 275: F938–F945
10. Lelièvre-Pégorier M, Vilar J, Ferrier M *et al*. Mild vitamin A deficiency leads to nephron deficit in the rat. *Kidney Int* 1998; 54: 1455–1462
11. Mendelsohn C, Batourina E, Fung S, Gilbert T, Dodd J. Stromal cells mediate retinoid-dependent functions essential for renal development. *Development* 1999; 126: 1139–1148
12. Batourina E, Cicconi E, Gilbert T, Mendelsohn C. Retinoids regulate embryonic kidney patterning. *J Am Soc Nephrol* 1999; 10 [Suppl]: 402A
13. Brenner B, MacKenzie H. Nephron mass as risk factor for progression of renal disease. *Kidney Int* 1997; 52: S124–S127