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Review

Relevance of proto-oncogenes as growth modulators in organogenesis of the mammalian embryonic kidney

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Introduction

Mammalian organogenesis constitutes a series of complex developmental processes which involve differentiation and rapid proliferation of pluripotent cells leading to the formation of a defined sculpted tissue mass, followed by a continuum of cell replication and terminal differentiation. These processes are modulated by various extracellular matrix (ECM) glycoproteins (Matrisian and Hogan, 1990), ECM receptors, *i.e.*, integrins (Pigot and Power, 1993), ECM degrading enzymes and their inhibitors (Kleiner and Stetler-Stevenson, 1993), cell adhesion molecules (CAMs) (Buxton and Magee, 1992), intracellular cytoskeletal proteins (Kries and Vale, 1993), growth factors or hormones and their receptors (Adamson, 1993), DNA-binding proteins/transcription factors (Latchman, 1993) and certain proto-oncogenes (Klein, 1993). The proto-oncogenes are growth regulatory genes normally expressed in mammalian cells, that with point mutations, amplification, chromosomal translocations, or DNA rearrangements acquire tumorigenic potential. They also encode for transmembrane receptor proteins of certain growth factors. These receptor proteins may either contain tyrosine or serine/threonine kinase intracellular catalytic domains, which conceivably endow proto-oncogenes with unique properties to regulate normal cell growth and proliferation subsequent to the binding of the putative ligand and phosphorylation of the receptor (Ullrich and Schleissinger, 1990, 1992).

The activities of the above described diverse group of macromolecules in embryonic development are intricately linked, and there is considerable variability in the cellular events and in the expression of various macromolecules, which are required predeterminants for the formation of individual organ systems. In other words, macromolecules with restricted genotypic or phenotypic expressions are relevant only to the morphogenesis of a particular organ system. Thus,"a morphogen is defined as a molecule that expresses its concentration gradient in a given tissue and alters the fate of target cells in a dose-dependent manner". This definition of a morphogen is applicable not only to proto-oncogenes, acting as receptors of certain ligands, but also to ECM macromolecules and their respective receptors, *i.e.*, integrins, cell adhesion molecules and growth factors and their receptors. In the same vein, differential concentration gradients of these macromolecules in various regions of the same developing tissue would induce different specific cellular events, thus adding complexity to the differentiation processes that ultimately leads to regional specialization within a given organ system. Finally, the magnitude of expression of various morphogenetic elements during fetal life would suggest that their function is specific for a given stage of a developing organ system. Although, the expression of morphogens is stage and tissue specific, a concerted coordination among the various macromolecules is critical for morphogenesis to proceed normally in the formation of a particular visceral organ.

Information concerning the role of morphogenetic modulators in development has been derived from *in vivo* knock out experiments in mice as well as *in vitro* culture systems applicable to mammary (Howlett and Bissell, 1993), prostate (Cunha, 1994) and salivary glands (Bernfield *et al.*, 1984), lung (Warburton *et al.*, 1993) and kidney (Saxen, 1987; Ekblom, 1992). With respect to morphogenesis, there are features common to these organ systems, *e.g.*, ephithelial-mesenchymal/ligand:receptor interactions, as well as differences in other aspects. These differences are somewhat unique to the development of the mammalian metanephros, and in the following section some of the features of renal development are briefly described.

General features of metanephric development

Renal development ensues following the successive appearance of the pronephros, mesonephros and metanephros as a craniocaudal wave of cellular differentiation in the nephrogenic cord lying alongside the nephric or Wolffian duct (Saxen, 1987; Ekblom, 1992). In mammals, the pronephros and mesonephros are vestigial elements of the nephrogenic cord, and their appearance is transitory, while the metanephros matures to form a permanent kidney. Interestingly, the mesonephros completely regresses in females, while the cranial mesonephric tubules, whose development is not regulated by the Wilms' tumor gene product (Sainio *et al.*, 1997) contribute to the formation of epididymal ducts in males.

Metanephrogenesis commences at day-11 of fetal life in the mouse and at about the 5th week of gestation in humans by sequential and reciprocal inductive interaction between the mesenchyme and the ureteric bud (Potter, 1972). During this process the ureteric bud, an epithelial-lined tubular structure arising from the Wolffian duct, interacts with the blastema, a loosely organized mesenchymal mass on the lateral aspect of the aorta in the most caudal segment of the nephrogenic cord. The interaction leads to differentiation of the mesenchyme into an epithelial phenotype, reciprocal inductive arborization of the ureteric bud and generation of nascent nephrons. It is of interest that mesenchymal differentiation continues even after the removal of the inducer, *i.e.*, ureteric bud, and differentiation can be maintained by soluble factors such as embryo extracts (Gossens and Unsworth, 1972; Bard *et al.*, 1996). On the other hand, soluble factors/ molecules, *e.g.*, anti-GD3 antibodies, can interfere in the inductive interaction even if the mesenchyme and the ureteric bud are in close contact with one another (Sariola *et al.*, 1988). Following inductive ephithelial-mesenchymal interaction, the nascent nephrons are formed by undergoing the following developmental stages, *i.e.*, condensate/ vesicle, comma-shaped body, S-shaped and pre capillary bodies (Fig. 1). The pre capillary bodies upon vascularization by the processes of vasculogenesis (*in situ* blood vessel formation) and angiogenesis (sprouting of pre-existing capillaries) form functioning mature glomeruli of the kidney (Saxen, 1987; Ekblom, 1992; Robert *et al.*, 1996).

To gain insights into the developmental dynamics of the metanephros, investigators have resorted to *in vitro* techniques, including organ or cell culture systems. Cell culture has been useful in studying the morphogenesis of tubules and the development of their epithelial junctions and polarity characteristics (Nathke *et al.*, 1993). To study the morphogenesis of the whole kidney and of the glomerulus and tubules, Grobstein (Grobstein, 1956, 1967) established an *in vitro* metanephric organ culture transfilter technique, which has been used widely with certain modifications for 4 decades by numerous investigators. In this culture system, various developmental stages, with the exception of vascularization of the metanephros, can be studied .The technique employs harvesting of either uninduced (day $10^{1/2}$) or induced (day $11^{1/2}$) metanephric mesenchyme, placing it on a microporous (~ 0.8 μm) filter and maintaining it in culture for 7-10 days. In this system, the uninduced

mesenchyme can be induced by placing ureteric bud or a heterologous inducer, *e.g.*, embryonic neural tissue, glued under the filter. The metanephric mesenchyme receives the inductive signals from the ureteric bud through the pores of the filter, perhaps by establishing pseudopodial cell-cell contacts (Saxen, 1987) or from the in growth of neuronal processes in the case of heterologous inducer (Sariola *et al.*, 1989), although induction by soluble factors is also conceivable (Perantoni *et al.*, 1995; Karavanova *et al.*, 1996). The induced mesenchyme goes through a series of differentiation events leading to glomerulo- and tubulo-genesis, while the uninduced mesenchyme may undergo apoptosis, and part of it develops into stroma or interstitium of the kidney. Finally, fundamental to the inductive neo-transformation of the mesenchyme are the molecules expressed at the ephithelial-mesenchymal interface, or ligands expressed in the mesenchyme and receptors in the ureteric bud epithelium which would be ideally suited for paracrine/juxtacrine interactions (Fig. 2). The concept of such ephithelial-mesenchymal juxtacrine/paracrine interactions, in nephrogenesis, was originally described for the extracellular matrix proteins and their receptors. Current data suggest that these interactions are applicable to proto-oncogenes, acting as receptors, and their ligands, as well as growth factors and their receptors (Birchmeier, C. and Birchmeier, W., 1993). In the context of such interactions, these diverse set of molecules exhibit a fair degree of interdependence in the regulation of the growth and development of the kidney. Interdependence between growth factors and ECM proteins is reflected by the fact that the latter may act as storage depots for certain growth factors or may contain growth factor-like domains (Ruoslahti and Yamaguchi, 1991). In such a scenario, the ECM would be expected to modulate the biologic activities of various growth factors, *e.g.*, TGF-β, while TGF-β in turn regulates the synthesis of ECM proteins (Sporn and Roberts, 1988). Similarly, *c-ret*, a proto-oncogene that regulates nephrogenesis (Schuchardt *et al.*, 1994) and ECM expression in metanephric tissues (Liu *et al.*, 1996), serves as a receptor for glial-derived nerve growth factor (GDNF) (Trupp *et al.*, 1996), the latter apparently is structurally related to members of TGF-β superfamily and plays a vital role in embryonic organ development (Moore *et al*, 1996; Pichel *et al.*, 1996; Sanchez *et al.*, 1996). Thus, the actions of this group of diverse molecules are intricately linked, and in this review the role of proto-oncogenes and transcription factors is discussed.

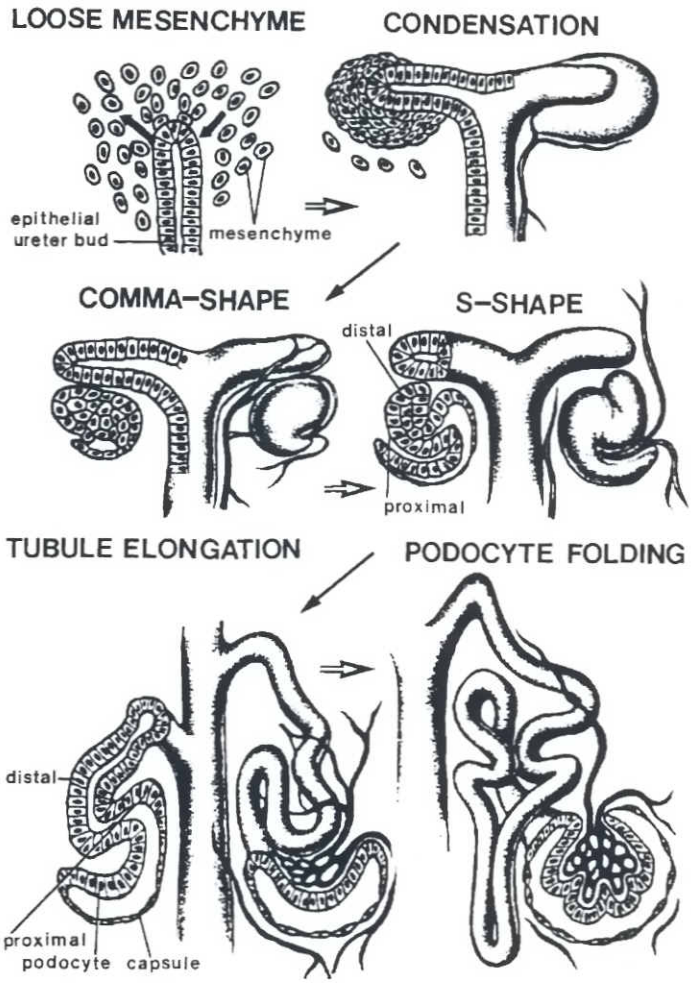

Fig. 1. Schematic drawing depicting various stages of differentiation of the nephron. *Formation of the nephron commences by interaction of the ureteric bud with loose metanephric mesenchyme. As a result, a condensate is formed, which goes through the comma-shape and S-shape-body stages. This is followed by tubule elongation to form the precapillary stage nephric unit. The precapillary unit is vascularized by an in-growth of extra-renal blood vessels leading to the formation of a mature glomerulus with an intricate capillary network (modified and reprinted with permission from Ekblom, P., 1992).*

Proto-oncogenes, transcription factors and suppressor genes

Following the conversion of metanephric mesenchyme to an epithelial phenotype, replication of differentiated cells occur under the influence of various growth factors and their receptors, some of the latter encoded by proto-oncogenes (Bard *et al.*, 1994). It should be noted that proto-oncogenes, in their mutated form, can induce aberrant proliferation and neoplasia. In addition, there are certain proto-oncogenes which can directly influence differentiation as well, *e.g.*, by *Wnt*, the term is an amalgam of the wingless (*wg*) and *int* genes (Nusse and Varmus, 1992). The *Wnts* have been regarded by some investigators as transcription factors and growth factors, and thus, it appears that differentiation and replication events are intricately linked in the biology of proto-oncogenes (Nusse and Varmus, 1992). The role of oncogenes in growth stems from transfection studies, *e.g.*, normal NIH3T3 cells infected with proto-oncogenes, exhibited accelerated growth and the acquisition of tumorigenic potential (Tsarfaty *et al.*, 1994). Furthermore, a number of proto-oncogenes, *e.g., c-bek, c-erb, c-fos, c-jun, c-max, c-met, c-mos, c-myc, c-myc/c-max, c-neu (c-erb B2), c-ras, c-ret, c-ros, c-sis, c-src and c-trk*, display a well-defined spatio-temporal expression in various *"normal"* embryonic tissues, suggesting that they also probably have a role in the early stages of mammalian development (Forrester *et al.*, 1992).

Proto-oncogenes related to the family of tyrosine kinase receptors

A number of proto-oncogenes transduce signals across the plasmalemma, and initiate a variety of intracellular events, which ultimately affect nuclear transcription (Yarden and Ullrich, 1988; Ullrich and Schlessinger 1990, 1992) . Basically, signal transduc-

tion is accomplished by protein products of the proto-oncogenes, which serve as high or low affinity receptors for ligands or growth factors. These receptors constitute transmembrane proteins containing intracytoplasmic domains which are endowed with intrinsic kinase catalytic activity for tyrosine phosphorylation. Following tyrosine phosphorylation, certain intracellular proteins are translocated to the nucleus, bind to DNA, enhance transcription and trigger cell replication. As in adult tissues, the proto-oncogenes and their ligands are co-expressed in certain embryonic tissues, *e.g.*, HGF/SF and *c-met* in the heart, condensing somites and neural crest cells, suggesting an "autocrine" mode of interaction (Andermarcher *et al.*, 1996). At times, the ligand: receptor interaction may be mediated in a "paracrine" or "juxtacrine" fashion, as exemplified by the expression of *c-ret* in the ureteric bud epithelium and its putative ligand GDNF in metanephric mesenchyme (Moore *et al.*, 1996, Suvanto *et al.*, 1996; Trupp *et al.*, 1996). This scenario is an apparent «recurring theme» in metanephric ephithelial-mesenchymal interactions, which may be largely mediated *via* the tyrosine kinase family of receptor proteins — the products of the following proto-oncogenes, *i.e.*, *c-erb, c-erb B2 (c-neu), c-met, c-ret, c-ros* and *c-trk*.

c-erb B2 is related to EGF receptor, and is a homolog of the rat *c-neu* gene (Bargmann *et al.*, 1986a; Yamamoto *et al.*, 1986). A single point mutation in *c-neu* gene renders it highly oncogenic (Bargmann et al, 1986b). *c-erb B2* encodes a 185-kDa transmembrane protein. The receptor protein is mainly expressed in respiratory, intestinal and renal epithelia, with higher levels of expression in embryonic than adult tissues. Its putative ligand is a soluble protein with M_r ~ 44 kDa that contains immunoglobulin and EGF sequence domains (Wen *et al.*, 1992), which can induce tyrosine phosphorylation of *c-neu*, and subsequent proliferation or differentiation of mammary epithelial cells. To stress its differentiation activity, the ligand has been named *neu* differentiation factor (NDF); an alternate name is heregulin (HRG) (Holmes *et al.*, 1992). The expression of NDF in the mesenchyme surrounding epithelia and *c-erb B2* in the epithelium are well-suited for paracrine interactions and a potential role in nephrogenesis (Birchmeier C. and Birchmeier W., 1993). The over-expression of *c-erb B2* in transgenic mice results in epithelial hyperplasia and pre-neoplastic lesions in kidney and lungs (Stocklin *et al.*, 1993).

trk family of proto-oncogenes encode high or low affinity tyrosine kinase receptors to which certain members of the nerve growth factor (NGF) family, including brain-derived neurotrophic factor (BDNF), NT-3, -4/5, bind and elicit their specific biological effects. Interestingly, a transmembrane protein, known as $p75^{LNGFR}$, binds to all the known members of the neurotrophin family with low affinity (Lee *et al.*, 1992). *trkA* is a receptor for NGF, and its co-expression with $p75^{LNGFR}$ leads to high affinity binding. *trkB*, a high affinity receptor, is activated by BDNF, NT-3 and -5, and *trkC* by NT-3. A hybrid receptor (*trk*/c-*ros*) transmits *c-ros*-specific signals in response to activation by NGF. NGF and its receptors are expressed in the collecting tubules and glomerulus, respectively. The low-affinity NGF receptor ($p75^{LNGFR}$), is expressed in the uninduced mesenchyme and developing podocytes of the S-shaped stage glomeruli (Durbeej *et al.*, 1993). *trkB* is expressed in the cortical mesenchyme that differentiates into stromal cells, and *trkC* is expressed in the medullary collecting ducts. Relevant to the role of NGF and its receptor in renal development, $p75^{LNGFR}$ antisense oligodeoxynucleotide inhibits ureteric bud branching morphogenesis (Sariola *et al.*, 1991), while sense oligodeoxynucleotide has been reported to have a minor effect (Durbeej *et al.*, 1993). Such renal deficits are not observed *in vivo* in mice lacking $p75^{LNGFR}$ (Lee *et al.*, 1992). However, this does not negate the relevance of neurotrophic factors in metanephric development since GDNF-deficient mice show renal hypo genesis (Moore *et al*, 1996; Sanchez *et al.*, 1996).

c-met serves as a receptor for urokinase plasminogen activator (*u-PA*) cleaved hepatocyte growth factor/scatter factor (HGF/SF) (Weidner *et al.*, 1993). The receptor is a heterodimer with α and β polypeptide chains, and its extracellular binding and tyrosine kinase domains are confined to the β-chain. The interaction of *c-met* with HGF/SF leads to diverse biological effects, including dispersion, motility, migration and proliferation of epithelial cells and effects on morphogenesis (Bhargava *et al.*, 1992). The dispersive activity of HGF/SF may be related to up-regulation of the activity of one of the ECM-degrading enzymes, *e.g.*, urokinase (*u-PA*) (Pepper *et al.*, 1992). However, it is the morphogenetic properties of HGF/SF which regulate renal tubulogenesis, define polarity characteristics, and modulate mammary gland ductulogenesis (Tsarfaty *et al.*, 1992; Santos *et al.*, 1993). HGF/SF and *c-met* are co-expressed in the metanephric mesenchyme, whereas *c-met* is exclusively expressed in the ureteric bud (Sonnenberg *et al.*, 1993), suggesting they play a role in renal development *via* a juxtacrine mode of interaction. Indeed, anti-HGF antibodies, which block the interaction of HGF/SF with *c-met*, inhibit the formation of epithelial condensates and ureteric branching morphogenesis in metanephros (Santos *et al.*, 1994; Woolf *et al.*, 1995), and the development of tubules in MDCK cells (Santos *et al.*, 1993). Although these *in vitro* data are provocative, the role of HGF/SF in renal development is not supported by knock-out mice experiments (Schmidt *et al.*, 1995).

The ***c-ret*** proto-oncogene is a member of the receptor protein tyrosine kinase family (Van der Geer *et al.*, 1994). It bears a sufficiently close resemblance to the receptors for EGF (*c-erb B2*), NGF (*c-trk*), HGF/SF (*c-met*) and the Steel factor (*c-kit*) that it can serve as an instructive model for understanding of the basic signaling pathways, mediated by receptor tyrosine kinases, essential for ephithelial-mesenchymal interactions in metanephric development (Pachnis *et al.*, 1993; Schuchardt et al 1994). The *c-ret* encodes a single transmembrane polypeptide with M_r ~ 120 kDa, and certain of its isoforms are expressed in various tissues (Iwamoto *et al.*, 1993). The extracellular segment of the polypetide contains motifs with sequence homology to cadherins, suggesting Ca^{++}-dependent homophilic interactions of the proto-Ret molecules. Binding to its putative ligand, GDNF, leads to the phosphorylation and activation of Ret protein, which in part may be mediated via a novel glycosylphosphatidylinositol-linked cell surface receptor, *i.e.*, GDNFR-a (Jing *et al.*, 1996; Treanor *et al.*, 1996). Interestingly, phosphorylation also can be induced by other growth factors, such as, TGF-α, IGF-I and HGF (Liu *et al.*, 1996). *c-ret* was originally discovered in patients with multiple endocrine neoplasia (MEN) types IIA and IIB (Mulligan *et al.*, 1993), and its expression is confined to the developing sensory- and autonomic neurons and genito-urinary systems. In the developing mouse metanephros, *c-ret* is expressed in the Wolffian duct at day 8.5-10.5, in the ureteric bud epithelium at day 11.0-11.5, and in the growing tips of the collecting ducts at day 13.5-17.5. Its constitutive expression in both

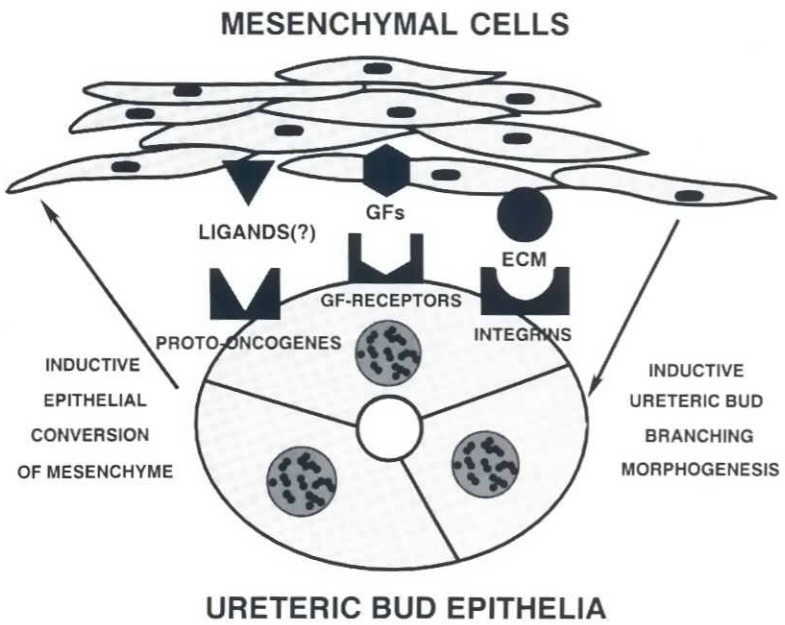

Fig. 2.Schematic drawing depicting the reciprocal induction of mesenchymal cells and ureteric bud epithelia in embryonic kidney. *The receptors of growth factors (GFs) and of extracellular matrix (ECM), i.e., integrins, are localized in the epithelial cells of the ureteric bud. The proto-oncogenes, such as, c-ret and c-ros, which function as tyrosine kinase receptors, are localized in the ureteric bud epithelium. Although the ligands of many proto-oncogenes are unknown, the ligand of c-ret is a glial-derived neurotrophic factor (GDNF), and is shown here to be expressed in the metanephric mesenchyme. Among the ECM proteins, some are expressed in the mesenchyme, while others are expressed at the epithelial:mesenchymal interface.*

the renal and neural tissues (the latter a known inducer) stimulated investigation of its role in metanephric development (Pachnis *et al.*, 1993). A targeted mutation in the mouse *c-ret* locus (*ret-K*$^-$) led to the deficiency of enteric neurons and varying degrees of hypoplasia or aplasia of the kidneys and ureters (Schuchardt *et al.*, 1994). More recently, *in vitro* co-culture experiments utilizing *ret-K*$^-$ or *ret-K*$^+$ mesenchyme and epithelium suggest that renal hypoplasia is due to defects in the ureteric bud, where *c-ret* is expressed prior to induction (Schuchardt *et al.*, 1996). As is true for *ret-K*$^-$ animals, GDNF-null mice, generated by homologous recombination in embryonic stem cells, also have an absence of enteric neurons and exhibit renal agenesis/dysgenesis (Moore *et al.*, 1996; Santos *et al.*, 1996); thus implying that the interaction of *c-ret*, expressed in the ureteric bud, with GDNF, expressed in the metanephric mesenchyme (Suvanto *et al.*, 1996), is essential for the development of the murine kidney.

c-ros, originally isolated from UR2 sarcoma virus, encodes a transmembrane protein with a predicted M_r ~ 260 kDa prior to post-translational modification (Riethmacher *et al.*, 1994). It is regarded as the mammalian homologue of the gene product of the *sevenless* from *Drosophila* (Chen *et al.*, 1991). The ligand for *sevenless* is the protein product of *bride of sevenless (boss)* gene, which spans the plasmalemma seven times, and is involved in the differentiation and morphogenesis of the compound eye of *Drosophila*. *c-ros* is transiently expressed during mouse embryogenesis, and its transcripts, like *c-ret*, have been localized to the tips of the ureteric bud branches (Sonnenberg *et al.*, 1991), suggesting a role in ephithelial-mesenchymal interactions in a "paracrine" manner while the ligand, although not well-characterized, is expressed in the mesenchyme (Riethmacher *et al.*, 1994). The role of *c-ros* was elucidated by *in vitro* experiments, where an inhibition of the ureteric bud branches and nephron formation was induced by inclusion of specific antisense oligos into metanephric culture medium (Durbeej *et al.*, 1993; Kanwar *et al.*, 1995). However, *c-ros*$^{-/-}$ male mice showed urogenital abnormalities confined only to the epididymis (Sonnenberg-Riethmacher *et al.*, 1996).

The other tyrosine kinase proto-oncogenes expressed during development belong to the *Src* family, and include *src*, *fyn* and *yes*. A deficiency of these genes, individually, does not adversely affect renal morphogenesis. However, in *fyn/yes* double mutant mice, post-natal degenerative changes and glomerulosclerosis were noted in the kidney (Stein *et al.*, 1994). However, the question as to which of these proto-oncogenes is most critical in nephrogenesis remains to be addressed. The effect of antisense oligodeoxynucleotides with sequences derived from the phosphotyrosine domains of a number of proto-oncogenes, *i.e., c-ret, c-ros, c-met, c-neu, c-trk* and *c-erb*, was assessed on the renal explants. Notable effects were observed only with *c-ret* (Fig. 3), which further emphasizes its extremely crucial role in metanephric development (Schuchardt *et al.*, 1994, 1996; Liu *et al.*, 1996).

Proto-oncogenes. Transcription factors

Besides tyrosine kinase receptors, there are other intracellular mitogen-activated protein kinases (MAPKs) which modulate growth signals that are transmitted to the nucleus (Lange-Carter *et al.*, 1993; Marx, 1993). In doing so, two distinct groups of cellular genes are induced. The first group, immediate-early genes (IEG) are induced in the absence of protein synthesis; whereas, the second group, late growth-regulated genes, are dependent on protein synthesis. Included in the IEG group are a number of proto-oncogenes, *e.g., c-myc, c-jun* and *c-fos*, and the recently discovered early growth response (EGR) family of genes (Gashler and Sukhatme, 1995). The EGR genes encode various transcription factors, which bind to targeted DNA sites and modulate cell proliferation. The *myc* proto-oncogene family, *i.e., c-myc (mycC), L-myc (mycL)* and *N-myc (mycN)* are expressed at high levels in the whole mouse embryo and in regenerating liver in partially hepatectomized rats, suggesting they play a role in normal growth

and differentiation (Downs *et al.*, 1989; Hirning *et al.*, 1991). Following translation, the *c-myc* onco-protein is rapidly transported into the nucleus where it heterodimerizes with the protein product of *c-max*. The *c-myc/c-max* complex then binds to specific DNA sequences and activates growth promoting genes (Koskinen and Alitalo, 1993). Conceivably, the process of heterodimerization is also applicable to IEGs, *e.g.*, between *c-jun* and *c-fos* (Gashler and Sukhatme, 1995). Among the IEGs, the *myc* family of proto-oncogenes have been thoroughly investigated in metanephric development (Mugrauer and Ekblom, 1991). *c-myc* mRNA is strongly expressed both in the uninduced and induced mesenchyme in the peripheral and middle cortex of the metanephros of the 16-day embryonic mouse. *N-myc* expression is low, and is localized in scattered clusters in the outer cortex. *L-myc* is mainly expressed in the epithelia of ureteric bud branches. These studies suggest that *c-myc* is involved in the early proliferative phases of metanephric development. The biological role of the *myc* family of proto-oncogenes in renal development was elucidated by insertional mutagenesis and homologous recombination experiments. The animals homozygous for *N-myc*⁻ showed slightly reduced morphogenesis of mesonephric tubules (Stanton *et al.*, 1992). In contrast, the results of *c-myc* insertional mutagenesis studies were quite dramatic. A fusion construct was generated by linking a noncoding DNA segment of mouse-mammary tumor virus (MMTV) with mouse *c-myc*. The transgenic mouse carrying the MMTV-*myc* recessive mutation developed aplasia or hypoplasia of one or both kidneys and limb deformities (*ld*) (Woychik *et al.*, 1985; Mass *et al.*, 1990). The recombinant proteins of the chimeric limb-deformity (*ld*) gene are referred to as «formins», whose action is variable in different tissues during development (Woychik *et al.*, 1990). The nuclear expression of formins is restricted to the pronephros and mesonephros, suggesting a role only in very early developmental processes.

Other signaling proteins, relevant to metanephric development, are encoded by *Wnt* genes. The *Wnt* genes encode secretory glycoproteins that act as multipotent factors, capable of inducing different biological responses in different cell types, a phenomenon reminiscent of several other growth factors, *e.g.*, FGFs, TGF-b and NGF (Nusse and Varmus, 1992). Apparently, they induce cell proliferation and differentiation by signaling through autocrine or paracrine routes, the latter conceivably involving their putative Wnt receptors (Nusse and Varmus, 1992). Some of the *Wnt* genes are expressed in the kidney, *e.g.*, *Wnt-4* (Stark *et al.*, 1994), while others are not expressed, *e.g.*, *Wnt-1* (Herzlinger *et al.*, 1994), but play a vital role as inducers in the differentiation of metanephric mesenchyme. For example, metanephric mesenchyme differentiates into a tubular epithelial phenotype, expressing E-cadherin, when co-cultured with NIH3T3 fibroblasts retrovirally transfected with *Wnt-1* cDNA and secreting Wnt-1 protein (Herzlinger *et al.*, 1994). These results are reminiscent of the data of experiments in which spinal cord was used as an inducer (Grobstein, 1956,1967). Interestingly, *Wnt-1* mRNA is expressed in the spinal cord, suggesting that Wnt-1 protein may be one of the constituents of spinal cord which dictates inductive conversion of the metanephric mesenchyme. The results of experiments with *Wnt-4*, are equally interesting. *Wnt*-4 is expressed in the metanephric mesenchyme and its early derivatives, *i.e.*, comma and S-shaped bodies. *Wnt-4*$^{-/-}$ mutant mice failed to form pretubular cell aggregates, suggesting a defect in mesenchymal-to-epithelial conversion. In addition, there was a loss of expression of other transcription factors, *i.e.*, *Pax-2* and *Pax-8*. It is noteworthy that the loss of expression of *Wnt-4* has also been observed in BMP-7$^{-/-}$ mutant mice with urogenital abnormalities (Dudley *et al.*, 1995; Luo *et al.*, 1995), reinforcing the relevance of *Wnt-4* in renal development.

Other transcription factor genes expressed during early renal differentiation include, homeobox *(Hox)* (Davis *et al.*, 1995) and paired-box *(Pax)* (Dressler *et al.*, 1993; Rothenpieler *et al.*, 1993). Interest has been focused on the *Pax* gene since *Krd* (kidney retinal defects) mice with deletion of the *Pax-2*, and spontaneous mutation at the *Sd* locus present in the vicinity of the *Pax-8* gene in *Danforth's short tail (Sd)* mice are accompanied by renal abnormalities (Gruss and Walther, 1992; Keller *et al.*, 1994). Similarly, transgenic mice over-expressing *Pax-2* exhibit disorganization of renal epithelial structures (Dressler *et al.*, 1993). Intriguingly, *Pax-2* translational blockade by antisense oligodeoxynucleotide *in vitro* results in the failure of mesenchymal cell aggregation and conversion into epithelial condensates, indicating an important influence during metanephric differentiation (Rothenpieler *et al.*, 1993). Both *Pax-2* and *Pax-8* genes are expressed in induced mesenchyme and early derivatives thereof, but never in the uninduced blastema. Although *Pax-5* has a high sequence homology with *Pax-2* and *Pax-8*, it is not expressed in the kidney (Asano and Gruss, 1992). Even though *Pax-2* is absent in the mesenchyme, gene disruption of *BF-2*, a specific transcription factor for stromal cells, is associated with loss of expression of *Pax-2* in tubular elements and with severe renal abnormalities, re-emphasizing the significance of ephithelial-mesenchymal interactions in renal development (Hatini, 1996). Compared to *Pax-8*, *Pax-2* appears earlier during development in comma-shaped bodies, ureteric bud branches and its derivatives, *i.e.*, collecting tubules, while the expression of *Pax-8* persists longer in S-shaped bodies before being down-regulated as nephron elements mature. The progression of *Pax-2* to *Pax-8* expression in the induced mesenchyme seems to require the *Wnt-4* gene (Stark *et al.*, 1994), suggesting that nephrogenesis is a multi-step process involving interactions between transcription factors and cell-signaling molecules. An example of gene interactions is the repression of transcription of *Pax-2* by the Wilms(tumor (*WT-1*) suppressor gene. The latter binds to *Pax-2* regulatory sequences upstream of the initiation codon in exon 1 (Ryan *et al.*, 1995). *Pax-2* can be transcriptionally activated by binding of EGR-1 to its promoter region for the *WT-1* recognition sites, suggesting that its activity is modulated by EGR-1 and *WT-1* (Sukhatme et al, 1988). Furthermore, *Pax-8* can transactivate the *WT-1* suppressor gene and its expression (Dehbi and Pelletier, 1996).

The *Hox* family include a number of genes which are expressed in embryonic and adult kidney tissues with an apparent concentration gradient in the medulla and cortex. Such gradients of expression are also seen in whole embryos where they are involved in regional specifications and patterning in the axial skeletal system. In this regard double mutant mice lacking *Hoxa-11* and *Hoxd-11*, genes normally expressed in the metanephric blastema, not only suffer from skeletal deformities but also have severe renal malformations (Davis *et al.*, 1995). The relevance of other homeoproteins expressed in the kidney, including hepatocyte nuclear factor-1α and -1β, await further investigations.

Suppressor genes

The discovery of suppressor genes was made by somatic cell genetic experiments in which fusion of tumorigenic and non-

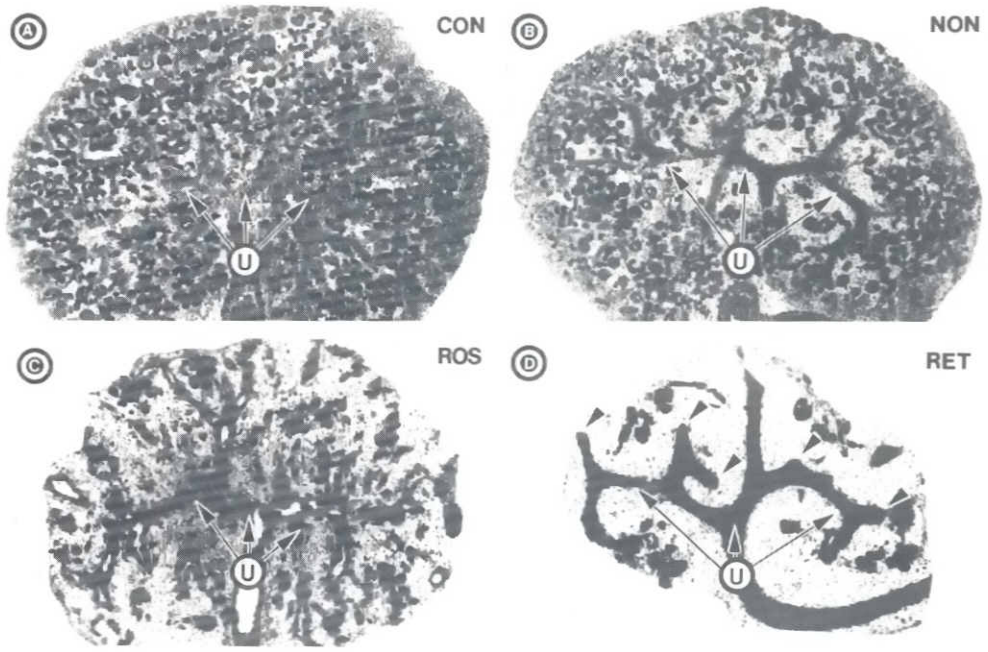

Fig. 3. Light photomicrographs of the untreated (A), nonsense ODN-treated (B), and *c-ros* and *c-ret* antisense ODN-treated (C and D) metanephric explants. *The c-ros antisense ODN caused blunting of the ureteric bud branches (U) with the formation of hollow tubular structures and a decreased population of the nephrons. In contrast, the c-ret antisense ODN induced severe dysmorphogenetic changes in the ureteric bud branches with blunting of their tips (arrow heads) and an almost complete ablation of nephron development and a notable reduction in the size of the embryonic kidney (reprinted with permission from Liu et al., 1996).*

tumorogenic cells resulted in a selective loss of neoplastic growth potential, but with the retention of certain properties of the cancerous cells (Levine, 1993). Suppressor genes encode proteins which usually repress transcription and inhibit cell growth. In certain instances, they may activate transcription as well. Thus, basically, they are transcription factors or nuclear proteins which negatively or positively regulate cell growth. The deletion of putative suppressor genes or «anti-oncogenes» is associated with several forms of cancer in man. The application of restriction fragment length polymorphism (RFLP) analyses of various neoplastic cells has led to the discovery of a number of suppressor genes in man, including *Rb*, *p53*, *WT-1*, *NF-1*, *APC*, *DCC* and *VHL* (Levine, 1993). The genes relevant to the present discussion are *p53* and *WT-1* (Maheswaran *et al.*, 1993; Haffner and Oren, 1995), which have potential functional interactions with one another, in that *WT-1* enhances transcriptional activation by *p53*, while wild-type *p53* seems to convert *WT-1* from a transcriptional activator to a transcriptional repressor. Conversely, increased expression of *WT-1* induces an increased steady-state level of *p53* and stability in its *trans*-activational properties. Moreover, *WT-1* inhibits *p53*-mediated apoptosis (Maheswaran *et al.*, 1995), a physiologic process which is an integral component of metanephric development (Koseki *et al.*, 1992) and is regulated by another set of proto-oncogenes, *i.e.*, *Bcl-2*, *BcL-x*$_L$, *Bax* and *Bak* (Chinnaiyan and Dixit, 1996). Although, there is an apparent intricate relationship between these two (*WT-1 and p53*) suppressor genes, *p53* transgenic mice exhibit fulminating apoptosis and hypo genesis of the kidneys. However, *WT-1* expression remains normal (Drummond *et al.*, 1994; Godley et al, 1996).

WT-1 was discovered in children with Wilms' tumor. It is a suppressor gene that spans about 50 Kb and has, at least, four splice variants (Drummond *et al.*, 1994). The amino terminus is enriched with proline, glutamine, serine and threonine residues — the structural motif found in other DNA binding proteins that regulate transcription (Madden *et al.*, 1993). The carboxy terminus contains KTS (lysine.threonine.serine) insertion in the four contiguous cysteine-histidine type zinc finger domains, which exhibit 70% homology to the early growth response gene (EGR) family and it binds to a DNA GC-rich nanonucleotide with the sequence GCGGGGGCG (Madden *et al.*, 1993). Embryologically, the tumor appears to arise in blastemal cells in the process of differentiation into epithelial forms (Pritchard-Jones and Fleming, 1991), and *WT-1* is expressed in the uninduced and induced (condensed) mesenchyme and in the developing S-shaped bodies and podocytes of the glomerulus (Pelletier *et al.*, 1991). *WT-1* mRNA is detectable during mid gestation, peaks 1-2 days after birth, and then declines to adult levels by the third week (Buckler *et al.*, 1991), suggesting a potential role in metanephric development. Its role in metanephric ephithelial-mesenchymal interactions is further emphasized in transgenic mice, where mutation in both the alleles for *WT-1* locus (*WT-1*$^{-/-}$) resulted in renal agenesis with failure in the outgrowth of the ureteric bud (Kreidberg *et al.*, 1993).

A question to be addressed is the mechanism(s) by which *WT-1* regulates renal organogenesis or growth. It may influence other proto-oncogenes or growth factors and their receptors expressed within the kidney or the Wilms tumor. In the latter, *N-myc* (Koskinen and Alitalo, 1993)], *Pax-2* [(Eccles *et al.*, 1992), N-CAM (Roth *et al.*, 1988), IGF-I (Gansler *et al.*, 1988), IGF-IR (Werner *et al.*, 1993), IGF-II (Drummond et al, 1992) and PDGF (Fraizer *et al.*, 1987) are expressed, and antibodies to IGF-IR have been shown to inhibit the growth of Wilms' tumor transplants in nude mice (Gansler *et al.*, 1989). Available evidence suggests that *WT-1* represses transcription of EGF-receptor, EGR-1, IGF-II and the PDGF-A and TGF-b1 genes (Drummond et al, 1992; Wang *et al.*, 1992; Englert *et al.*, 1995; Gashler and Sukhatme, 1995). The prevalent notion is that the IGF-II is the physiological target for both EGR-1 and *WT-1*, the transcription factors that compete for the same DNA binding motif, 5'-GCGGGGGCG-3' (Madden *et al.*,

1993). Conceivably, a "balance" between the EGR-1 (a positive regulator) and *WT-1* (a negative regulator) is required for metanephric growth to proceed normally (Gashler and Sukhatme, 1995). Finally, a second *WT-1* binding sequence (5'-TCCTCCTCCT CCTCTCC-3'), noted in the promoters of several other growth-related genes, *i.e.*, EGF-receptor, *c-myc*, *Ki-ras*, tumor growth factor β_3 and insulin receptor, may also be relevant to the regulation of various developmental processes (Wang *et al.*, 1993). The fact that insulin and its receptor, that mainly regulate glucose metabolism, are also expressed in the early metanephros and heavily influence its development (Liu *et al.*, 1997), further enforces the relevance of *WT-1* in renal organogenesis.

SUMMARY

During embryonic life, renal morphogenesis is characterized by a defined period of intense cellular activity, inductive-transformation of undifferentiated cells to polarized epithelia, ingrowth of capillaries into an intricate parenchymal ephithelial-mesenchymal mass, and finally the maturation into an organ with diverse structural and biological functions. It should be emphasized that the interactions between various proto-oncogenes, serving as receptors, and the growth factors and other morphogenetic modulators, *e.g.*, ECM glycoproteins, are required for proper ephithelial-mesenchymal interactions essential to the process of nephrogenesis. A "*balance*" between the activities of these diverse group of macromolecules, whether essential or redundant, is needed to orchestrate the proper cell signals and proliferative responses to assure the progression of normal organogenesis (Pardee, 1987). Finally, in spite of the enormous wealth of data in the literature, the process of renal development is so complex that a clear picture has yet to emerge of the precisely coordinated sequential events that result in the formation of the mature functioning kidney.

KEY WORDS: *renal development, proto-oncogenes, transcription factors, suppressor genes, growth factors, extracellular matrix*

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