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Nephron induction

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Abstract
One of the most remarkable transformations of cells during organogenesis is the epithelial transformation of nephrogenic mesenchyme to secretory nephrons. During recent years, gene targeting and organ culture approaches have been used efficiently to resolve key molecules involved in this multistage process. Nephrons are induced by the tips of the branching ureteric bud that later forms the collecting duct network. The first signal in nephron induction is obviously maintaining the mesenchyme; the second enhances cell proliferation and brings together the set of cells that contribute to one single nephron. This stage is characterized by two types of condensations (first the cap stage and then pre-tubular condensation). The final step, epithelial transformation, is a cell-autonomous process. Although the molecular cascade in nephron induction is being resolved in the rat, the same signals seem to work less efficiently in the mouse. In the rat, fibroblast growth factor-2 maintains the nephrogenic mesenchyme; leukaemia inhibitory factor together with transforming growth factor β-2 induce its condensation; and autocrine secretion of Wnt-4 converts it to epithelium.

Keywords: fibroblast growth factor-2; inductive tissue interaction; leukaemia inhibitory factor; nephron induction; transforming growth factor β-2

Nephron induction is a multistage process

Nephron induction is initiated when the ureteric bud invades the nephrogenic mesenchyme (MM). In response to the bud, MM undergoes rapid cell division, it condenses, it down-regulates apoptosis and it gradually undergoes mesenchyme to epithelium transition. Nephron induction continues over much of embryogenesis. If the MM is surgically microdissected from the bud and cultured in organ culture without an inducer tissue, the cells are lost within a few days [1–3].

Morphological and genetic evidence has shown that, under the inductive influence of the ureteric bud, the MM differentiation is a multistage process. The MM is pre-programmed for tubulogenesis and is the only mesenchyme in the embryo that can form kidney tubules [4]. Initially, the whole MM condenses around the invading ureteric bud and escapes the apoptotic default pathway. This early mesenchymal condensate, the cap stage, should not be confused with the subsequent stage, the pre-tubular condensations that precede intimately the epithelial transition to nephrons. In a recent review, we have defined this structure as follows [5]: ''This cap-like structure surrounding the tip should not be confused with the small pretubular condensates that form a little later at the proximal edge of the cap and that will subsequently undergo epithelial transition on the way to becoming nephrons. Some authors have named this initial cap condensation a primary condensate. However, because most cap cells do not contribute to the pretubular condensates and because the cap is seen not only in the early kidney rudiment, but also at each duct tip through much of development, we propose that the term primary condensate should be avoided. It is less misleading to refer to these cell aggregates simply as 'caps'.''

In the mouse, metanephric mesenchymal cells convert to nephron epithelium in 48 h, and, after 24 h contact with an inducer, the epithelialization process becomes autonomous, and no longer requires the influence of an inducer. Once initiated, nephrogenesis continues for a considerable amount of time and only ceases when the last mesenchymal cells have been induced. This occurs during late embryogenesis in man and soon after birth in rat and mouse, and it seems to be due to an inability of the MM cells to divide sufficiently to maintain themselves so that they are finally all triggered for nephrogenesis.

Molecular basis of nephron induction

Isolated rat but not mouse MM undergoes full epithelial differentiation, if fibroblast growth factor-2

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Table 1. Signal substances or chemicals implicated in the maintenance of metanephric mesenchyme or in the induction of nephrogenesis *in vitro*

Protein, chemical or extract	Response type in MM
FGF-2	Maintenance
LIF	Epithelial differentiation (after FGF-2 priming), efficient only in rat
Oncostatin M	Epithelial differentiation (after FGF-2 priming), efficient only in rat
Wnt:s	Epithelial differentiation in co-culture with Wnt-transfected cells
BMP-7	Maintenance. Epithelial differentiation also proposed, other studies have not verified this effect
Pituitary extract	Epithelial differentiation (when combined with FGF-2)
Ureteric bud culture concentrate	Epithelial differentiation (when combined with FGF-2)
Lithium	Differentiation up to the prertubular condensation stage

(FGF-2) is combined with a conditioned medium from ureteric bud cell cultures, or if the FGF-2 pulse is followed by leukaemia inhibitory factor (LIF) treatment [6–8]. FGFs and bone morphogenetic proteins (BMPs) act synergistically during nephrogenesis to maintain the competence of the MM [9]. The *in vivo* significance of these *in vitro* observations and, in particular, the reason for different responses to inducer molecules in rat and mouse remains to be determined.

Some other molecules have also been implicated in nephron induction, such as transforming growth factor β-2 (TGF-β-2) [10] and BMP-7 [11,12]. Although BMP-7 has been implicated in nephron induction [11], it maintains renal cells rather than inducing their differentiation [9,13,14]. TGF-β-2-deficient mice show a range of renal defects from hydronephrosis to unilateral renal aplasia [10], suggesting a role in nephron differentiation. Recent organ culture studies have verified that TGF-β-2 acts in synergy with FGF-2 and LIF. If all these growth factors are used in concert, nephrons are induced *in vitro* as efficiently and as quickly as by spinal cord or the normal inducer, the ureteric bud [15].

In vivo vs in vitro

Although this triplet of signalling molecules is clearly inducing kidney tubules in organ culture efficiently, it has not been shown that they would represent the physiological inducers, at least not in the mouse. For instance, gene-targeted mice deficient for LIF receptor have kidneys, although they are smaller than normal [16]. The variable phenotype of TGF-β-2 and FGF-2 null mice might represent redundancy, and our ongoing studies of FGF receptor I hypomorphic mice clearly indicate that its ligands, including FGF-2, affect kidney morphogenesis (unpublished data and [17]). Another unsolved question concerns the role of Wnt-4. It is clearly an autocrine epithelializing signal, and the lack of Wnt-4 stops kidney differentiation at the condensation stage [18,19]. However, cells transfected by different Wnt genes can induce full epithelial differentiation of isolated MM [19,20]. Until the Wnt proteins have been purified and their inductive role has been tested, the most plausible explanation is that the Wnt-transfected cells secrete other growth factors that, in concert with the transgene product, might act as inducers.

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