Secreted Molecules in Metanephric Induction

THOMAS J. CARROLL and ANDREW P. McMAHON
Department of Molecular and Cellular Biology, Biological Laboratories, Harvard University, Cambridge, Massachusetts.

Abstract. Nearly 50 yr ago, Clifford Grobstein made the observation that the ureteric bud induced the nephrogenic mesenchyme to undergo tubulogenesis. Since that discovery, scientists have attempted to characterize the molecular nature of the inducer. To date, no single molecule that is both necessary and sufficient for nephric induction has been identified. Because of recent insights regarding the role of several secreted molecules in tubulogenesis, it has become necessary to revise the classic model of metanephric induction. The studies of the classic ureteric inducer performed to date have most likely been characterizations of a mesenchyme-specific inducer, Wnt-4, and its role in tubulogenesis. Ureteric induction most likely involves a series of distinct events that provide proliferative, survival, and condensation signals to the mesenchyme, integrating the growth of the ureteric system with tubulogenesis.

The developmental biologic processes of the kidney have been the subject of intense study for more than 100 yr (for review, see reference (1)). All three vertebrate kidney types (pronephros, mesonephros, and metanephros) are derivatives of a region of the embryo known as the intermediate mesoderm. In mice, a portion of the mesonephric duct, known as the metanephric bud, branches dorsally approximately 10.5 d after coitus and invades the caudal-most portion of the nephrogenic mesenchyme. The bud branches several times while growing peripherally and eventually forms the collecting duct system of the mature kidney. Shortly after invasion by the bud, the mesenchyme juxtaposed to the bud condenses, undergoes an epithelial transformation, and fuses with the duct, forming the metanephric tubule. The tubule elongates and proceeds through a series of intermediate forms, known as the comma- and S-shaped bodies, before ultimately forming the mature renal tubule. The proximal-most region of the tubule forms a specialized structure, i.e., the glomerulus, which is vascularized and is the primary filtration apparatus of the organ. Mature murine kidneys consist of approximately 0.5 million precisely arranged functional units, or nephrons, connected to the collecting duct system. Because of the importance of the kidney in maintaining proper blood chemistry values, the correct arrangement and number of nephrons are essential.

It has been nearly 50 yr since Clifford Grobstein discovered that interactions between the ureteric bud and the adjacent mesenchyme affect the normal development of the metanephric kidney (2–6). Grobstein revealed that, if the ureteric bud is separated from the metanephric mesenchyme at an early stage of development, then the mesenchyme fails to undergo tubulogenesis. The conclusion drawn from this discovery was that the ureteric bud induces tubulogenesis within the surrounding mesenchyme. During further investigation, it was discovered that a number of tissues, including, most notably, a dorsal portion of the embryonic spinal cord, are able to substitute for the ureter in this inductive interaction. Although the spinal cord certainly plays no role in the normal induction of the mesenchyme, it was assumed that this functional mimicry is attributable to the expression of the endogenous inducer within the spinal cord tissue. In fact, the spinal cord seems to be a far more effective inducer than the ureter itself and, since this discovery, it has been used in place of the ureter in the majority of studies on metanephric induction. For example, observations that the inductive event could take place across a filter (suggesting that the inducer was a secreted molecule) but required a large enough pore size to allow cell/cell contact (suggesting that the secreted molecule was closely associated with the cell membrane) were made using spinal cord as the inductive source.

In recent years, developmental biologists have sought the molecules responsible for a number of inductive signals that were first identified embryologically, including the ureteric inducer. On the basis of the initial characterizations made by Grobstein, candidate molecules for the kidney inducer should satisfy a number of criteria (7). First, and most important, a candidate molecule must be expressed in the ureteric bud at the time of induction, i.e., 10.5 d after coitus in mice. Second, a candidate molecule must be a secreted factor that is closely associated with the cell membrane. Finally, if the molecule is truly involved in tubule induction, then it must be both necessary and sufficient for normal induction.

This model makes the assumption that there exists a single inducing molecule, which is the simplest hypothesis in the absence of contradicting data. However, to date, no single molecule that satisfies all of these criteria has been identified. Several secreted molecules, including members of the bone morphogenetic protein, fibroblast growth factor (FGF), insulin-like growth factor, and Wnt families of growth factors, are

Correspondence to Dr. Andrew P. McMahon, Department of Molecular and Cellular Biology, Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138. Phone: 617-496-3757; Fax: 617-496-3763; E-mail: amcmahon@biosun.harvard.edu
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expressed in the invading ureteric bud; however, none of them are capable of inducing tubulogenesis and thus cannot be the classic inducer (for review, see reference 8). Although it was recently demonstrated that members of the wingless/int family of secreted glycoproteins are capable of inducing tubulogenesis in vivo (9), to date only one Wnt (Wnt-11) that is expressed in the tip of the ureteric bud during the inductive phase has been identified (10). Unfortunately, as discussed below, this molecule is not sufficient to induce tubulogenesis in vitro. If a single inductive factor exists, it cannot be one of the already characterized Wnt proteins. Furthermore, current data suggest that the Wnt signal originates not from the ureter but rather from the metanephric mesenchyme (11). In light of these data, it seems appropriate to reevaluate the nature of the metanephric inducer and the role of secreted proteins in the process of induction.

Role of Wnt Proteins in Kidney Development

The Wnt proteins have been demonstrated to play essential roles in a number of developmental processes in a wide range of organisms (for review, see reference 12 or www.stanford.edu/~rmusse/pathways/allcomp.html). The discovery that cell lines expressing Wnt-1 are capable of inducing metanephric mesenchyme to undergo tubulogenesis (9) led to the hypothesis that Wnt proteins may function as the classic metanephric inducer. In fact, the spinal cord, which is capable of substituting for the endogenous inducer, expresses several Wnt proteins, providing further circumstantial evidence that this important family of molecules may be involved in metanephric induction. Because Wnt-1 is not expressed in developing kidneys, it has been hypothesized that this molecule mimics another Wnt in in vitro experiments. To date, four Wnt proteins that are expressed in the developing metanephros have been identified, i.e., Wnt-4, -6, -7b, and -11 (10). Because Wnt-6 is expressed at extremely low levels and is thought not to play a major role in kidney development, it is not further discussed. We concentrate on the prospective roles of Wnt-4, -7b, and -11 in induction and later nephric development.

Wnt-4

The first Wnt shown to be involved in kidney development was Wnt-4 (13). Wnt-4 is expressed in the aggregating metanephric mesenchyme at 11.5 d after coitus, shortly after ureteric bud invasion. Targeted ablation of this gene results in embryos with severely hypoplastic kidneys. In the absence of Wnt-4, the metanephric mesenchyme condenses normally but fails to aggregate into pretubule clusters and undergo tubulogenesis. Morphologic and molecular marker analyses suggest that Wnt-4 is involved in the transition of tubular mesenchyme to epithelium. Interestingly, the ureteric epithelium of mutant kidneys undergoes several rounds of branching, suggesting that tubulogenesis is not required for this process. The expression patterns and phenotypes of Wnt-4 mutants suggest that Wnt-4 is a mesenchymal factor that acts downstream of the initial inductive events. Therefore, Wnt-4 cannot be the ureteric inducer.

Wnt-7b

A second family member that is expressed in the developing metanephros is Wnt-7b (10). Unlike Wnt-4, Wnt-7b is expressed within the epithelium of the ureteric bud. However, the time at which Wnt-7b mRNA can first be detected within the ureteric epithelium (13.5 d after coitus) is not consistent with this molecule playing a role in induction. Furthermore, Wnt-7b mRNA is excluded from the tips of the ureter, the region thought to possess inductive properties, further diminishing the possibility of a role for this molecule in the inductive process. Preliminary genetic analyses suggest that Wnt-7bh plays a role in duct maintenance or growth (Carroll, Pepicelli, & McMahon, unpublished observations), rather than induction.

Wnt-11

A search for Wnt genes expressed in the developing kidney revealed the previously uncharacterized Wnt-11 (10). The expression pattern of this gene was particularly intriguing to nephrologists. Wnt-11 is expressed within the mesonephric duct just before metanephric induction. As the ureteric bud invades the metanephric blastema, Wnt-11 expression intensifies within the leading edge of the ureteric bud. As the ureter branches, Wnt-11 expression is maintained at the tips of each branch but is lost in the intervening epithelium. Wnt-11 continues to be expressed at the tips of the ureter as they move toward the cortex of the developing kidney, which is a region of continuing induction.

Wnt-11 expression closely matches that expected for a molecule involved in tubule induction. However, when cell lines expressing Wnt-11 were co-cultured with metanephric mesenchyme, they failed to induce tubulogenesis (11). Wnt-11 therefore fails to satisfy at least one of the criteria (sufficiency) for a metanephric inducer. It seems unlikely that this molecule plays a role in induction. Quite surprisingly, in this same assay it was found that cell lines expressing Wnt-4 were capable of inducing tubulogenesis. This result is surprising in light of the fact that Wnt-4 was previously shown to act downstream of the ureteric signal. Furthermore, it has been shown that metanephric mesenchyme itself is not capable of inducing tubulogenesis (so-called homeogenetic induction) (14). The fact that a gene that is expressed within the metanephric condensations and acts downstream of the ureteric signal is capable of inducing tubulogenesis is quite unexpected.

Signal Relay in Tubulogenesis

As mentioned above, nearly all of the characterizations of metanephric induction have been performed using spinal cord as the inductive tissue, under the assumption that this tissue mimics the endogenous inducer. Because the Wnt molecules behave identically to the spinal cord in this in vitro assay, it has also been assumed that they mimic the ureteric signal. However, the ability of Wnt-4 to induce tubulogenesis necessitates a reevaluation of these findings.

If the Wnt molecules truly mimic the ureteric inducer, then co-culture of a Wnt-expressing cell line with Wnt-4 mutant mesenchyme should not induce tubulogenesis (because Wnt-4 has been shown to produce its effect downstream of the ure-
teric signal). Quite surprisingly, this was not the case. Several Wnt-expressing cell lines could induce tubulogenesis in Wnt-4 mutant mesenchyme, suggesting that the Wnt proteins produce their effects at the level of or downstream of Wnt-4 (11). Therefore, it seems that the Wnt proteins do not mimic the classic inductive signal at all. The characteristics of the classic inducer were defined using spinal cord as the inductive tissue. The spinal cord, like the Wnt proteins, was assumed to mimic the endogenous ureteric signal. The ability of the spinal cord to induce tubulogenesis might be attributable to the fact that it expresses several Wnt proteins (including Wnt-4), rather than the possibility that it expresses the endogenous inducer. To investigate this possibility, the inductive properties of the spinal cord were reexamined (11). If the spinal cord mimics a ureteric signal, its inductive abilities should be blocked by Wnt-4 mutant mesenchyme. However, if the spinal cord is simply mimicking the Wnt signal, then it should be able to induce tubulogenesis in Wnt-4 mutant mesenchyme. Rather surprisingly, the latter hypothesis seems to be correct. Spinal cord is capable of inducing tubulogenesis just as well in Wnt-4 mutant mesenchyme as in wild-type mesenchyme. This finding strongly suggests that the spinal cord signal is acting at the level of or downstream of Wnt-4 within the metanephric mesenchyme. This further suggests that most studies of the nature of the metanephric inducer that have been performed to date have actually characterized a mesenchymal inducer, Wnt-4. Metanephric induction seems to involve a series of events that originate in the ureter but includes mesenchyme-specific signals.

**Ureteric Inducer 2000**

The data reviewed here suggest that much of what we know (or thought we knew) regarding the nature of the ureteric inducer is probably incorrect. The studies performed to date have most likely characterized Wnt-4 and its role in tubulogenesis. These recent discoveries present nephrologists with a number of unanswered questions. What do we know regarding the molecular nature of the ureteric signal? Because we must ignore much of the previous data (because it was obtained using spinal cord), the only fact we know definitely is that the ureter is necessary for tubulogenesis. It remains unclear whether the ureter itself actually induces tubulogenesis. There are several alternative explanations for the lack of tubulogenesis after removal of the ureter. For example, the ureter could provide survival and/or proliferative signals to the metanephric mesenchyme (dead cells do not form tubules). This is supported by the observations that mesenchyme cultured in the absence of an inducer undergoes apoptosis (15) and some signals produced by the ureter, including bone morphogenetic protein-7, promote survival and growth of the mesenchyme but do not induce tubulogenesis (16,17). However, other factors produced by the ureter, including β-FGF (18), cause cultured metanephric mesenchyme to condense, suggesting that this structure provides more than just survival or proliferative signals. These data suggest that metanephric induction is not a single event. Further support for this notion is derived from several recent studies. It has been demonstrated that an unidentified factor (or factors) from a ureteric bud cell line, in combination with β-FGF and transforming growth factor-α (TGF-α), supports full differentiation of cultured metanephric mesenchyme (19,20). Recently, Barasch et al. (21) demonstrated that leukemia inhibitory factor (LIF), which by itself is unable to induce tubulogenesis, can induce full differentiation when supplemented with β-FGF and TGF-α, suggesting that it may be the unidentified ureteric factor. Interestingly, although LIF is expressed in the ureter, it seems to act downstream of the primary inductive signal, inasmuch as markers of the initial inductive response (Pax-2 and Wnt-4) are expressed in cultured mesenchyme in its absence (21). This observation correlates well with the fact that β-FGF by itself can cause condensation but not tubulogenesis of metanephric mesenchyme (18). However, it is surprising that the expression of Wnt-4 in mesenchyme cultured with β-FGF and TGF-α does not lead to tubulogenesis, as would be expected from the *in vitro* experiments. There are clearly regulatory events that we do not now understand, and further investigation is needed.

**Conclusion**

Currently available data support a multifactorial process for metanephric induction. Tubulogenesis most likely requires multiple sequential inductive signals from the ureter, in combination with feedback from the mesenchyme to the ureter (Figure 1). After the initial inductive event (possibly involving β-FGF), the mesenchyme is signaled to condense. Further differentiation (tubulogenesis) requires additional signals, which, at least in *in vitro*, can be provided by the LIF molecule. Placing Wnt-4 within this system is more difficult. The experiments by Barasch et al. (21) suggest that Wnt-4 is downstream of the FGF-like signal but upstream of LIF. However, at least *in vitro*, Wnt-4 alone is sufficient to induce tubulogenesis in the absence of additional ureteric factors, seemingly in contradiction to these results. Further investigation is certainly needed to establish the organizational relationships of these molecules during kidney development. It would be interesting to test the ability of Wnt-4 to induce tubulogenesis in LIF mutant mesenchyme. Unfortunately, neither LIF nor its receptors manifest a severe kidney phenotype when mutated (perhaps because of molecular redundancy), precluding these experiments. However, assessments of the ability of LIF to induce tubulogenesis in Wnt-4 mutant mesenchyme can be performed and should help us to better understand this series of events.

Regardless of how these signals relate to each other, it seems quite clear that multiple signals from the ureteric bud and the metanephric mesenchyme cooperate to integrate the survival, proliferation, and condensation of the metanephric blastema with ureteric branching. This sequence leads to a tightly regulated and precisely controlled process of nephric morphogenesis. With increased understanding, we should be able to differentiate each of these events at both the molecular and morphologic levels. The next several years should provide us with evidence that will further clarify these processes. Whatever the future brings, it seems certain that the classic one-signal model for metanephric induction is greatly oversimplified. The future holds great promise for the discovery of unique
factors and further understanding of previously identified factors in ureteric and mesenchymal signaling. With the precise roles of several Wnt molecules still unknown, it seems likely that this important family of molecules will be demonstrated to play additional roles in the complex series of events that leads to the development of the metanephros.

References