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Review

Hormonal, cellular, and molecular control of prostatic development

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Abstract

The prostate is a male accessory sex gland found only in mammals that functions to produce a major fraction of seminal fluid. Interest in understanding the biology of the prostate is driven both by the fascinating nature of the developmental processes that give rise to the prostate and by the high incidence in humans of prostatic diseases, including prostatic adenocarcinoma and benign prostatic hyperplasia. This review summarizes the current state of knowledge of the cellular and molecular processes that control prostatic development. Insight into the mechanisms that control prostatic development has come from experimental embryological work as well as from the study of mice and humans harboring mutations that alter prostatic development. These studies have demonstrated a requirement for androgens throughout prostatic development and have revealed a series of reciprocal paracrine signals between the developing prostatic epithelium and prostatic mesenchyme. Finally, these studies have identified several specific gene products that are required for prostatic development. While research in recent years has greatly enhanced our understanding of the molecular control of prostatic development, known genes cannot yet explain in molecular terms the complex biological interactions that descriptive and experimental embryological studies have elucidated in the control of prostatic development.

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Overview of prostatic development and structure

Prostatic development

Growth and development of the prostate begin in fetal life and are complete at sexual maturity. The prostate develops from the urogenital sinus (UGS), a subdivision of the cloaca. The UGS is a midline structure with an endodermally derived epithelial layer surrounded by a mesodermally derived mesenchymal layer. The UGS is found just caudal to the neck of the developing bladder (Fig. 1A). The UGS arises in both male and female mice at approximately 13 days postconception (dpc) and in humans at about 7 weeks of gestation (23 mm; Hamilton et al., 1959). The male and female urogenital sinuses are morphologically indistinguishable until about 17.5 dpc in the mouse and 10–12 weeks in humans, at which time prostatic morphogenesis commences in a process that is both initiated by and dependent on circulating androgens produced by the fetal testes.

The initial event in prostatic morphogenesis is the outgrowth of solid epithelial buds from the urogenital sinus epithelium (UGE) into the surrounding urogenital sinus mesenchyme (UGM). Initially, prostatic buds are solid cords of epithelial cells that grow into the UGM in a precise spatial pattern that establishes the lobar subdivisions of the prostate [(Timms et al., 1994a); rodent reviewed in Cunha et al. (1987); human (Kellokumpu-Lehtonen, 1985; Lowsley, 1912)]. In rodents, most of the prostatic ducts are unbranched at birth. However neonatally, as these ducts elongate within the UGM, they begin to bifurcate and send out side-branches. Ultimately, the process of branching morphogenesis in rats and mice will give rise to three distinct bilaterally symmetrical prostatic lobes: the anterior prostate (AP; also known as the coagulating gland), the dorsolateral prostate (DLP), and the ventral prostate (VP; Fig. 1B). The ducts of each of these prostatic lobes have a

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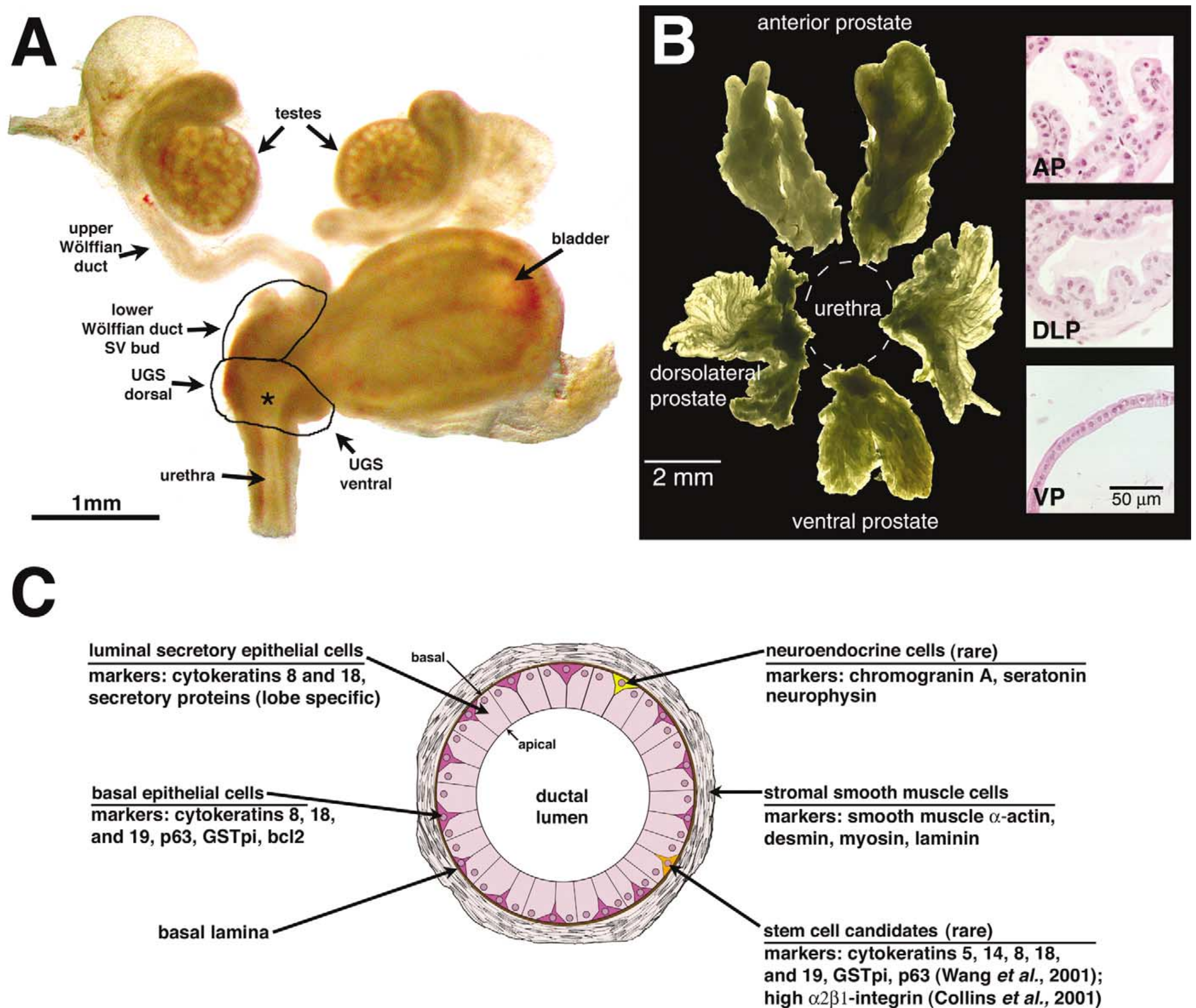

Fig. 1. Morphological and cellular features of the prostate gland. A portion of the urogenital tract from a male embryonic day 16.5 mouse is shown (A). The prostate develops from the urogenital sinus (UGS), which is located at the base of the developing bladder. At this embryonic stage, the urogenital sinus epithelium is visible as a dilation of the urethra (lighter area containing *) surrounded on the dorsal and ventral sides by condensed urogenital sinus mesenchyme (darker areas). The lobes of the adult mouse prostate are shown (B) together with hematoxalin and eosin-stained sections of prostatic ducts from each lobe (B, inset micrographs). Each lobe has a distinct shape and histologic appearance. A diagram of a ductal cross-section is shown (C) with labels indicating cell types that are present in prostatic ducts including luminal secretory epithelial cells, basal epithelial cells, neuroendocrine cells, stromal smooth muscle cells, and stem cell candidates. Beneath the label for each cell type is a list of differentiation markers commonly used to distinguish these cell types. In the case of stem cell candidates, two studies have associated expression of either a set of markers including cytokeratins 5, 14, 8, 18, and 19, GSTpi, and p63 (Wang, 2001) or high expression of $\alpha2\beta1$-integrin (Collins, 2001) with rare basal cells proposed as prostatic epithelial stem cell candidates. It is not currently clear whether the cell type or types from these two studies are identical. Scale bars for photos and micrographs are shown in (A) and (B).

characteristic branching pattern (Sugimura et al., 1986a). Concurrent with the process of ductal branching morphogenesis, epithelial and mesenchymal/stromal cytodifferentiation occurs in the first 2–3 weeks after birth in rats and mice. Epithelial cells of the developing, solid prostatic buds are characterized by coexpression of cytokeratins 5, 8, 14, and 18, and p63 (Wang et al., 2001). These solid cords elongate into the surrounding mesenchyme as a result of intense proliferative activity at their tips (Sugimura et al., 1986b). Ductal canalization is initiated in the solid epithelial cords from their urethral terminus and proceeds distally toward the ductal tips. As the solid epithelial cords canalize, the epithelium reorganizes into two distinct cell populations. Basal epithelial cells become localized along the basement membrane to form a discontinuous layer of cells expressing cytokeratins 5 and 14, and p63 in the rat and mouse. Concomitantly, tall columnar luminal cells, which express cytokeratins 8 and 18, differentiate and line the ductal lumina (Hayward et al., 1996a). By this process, the epithelium of the solid epithelial buds (expressing both

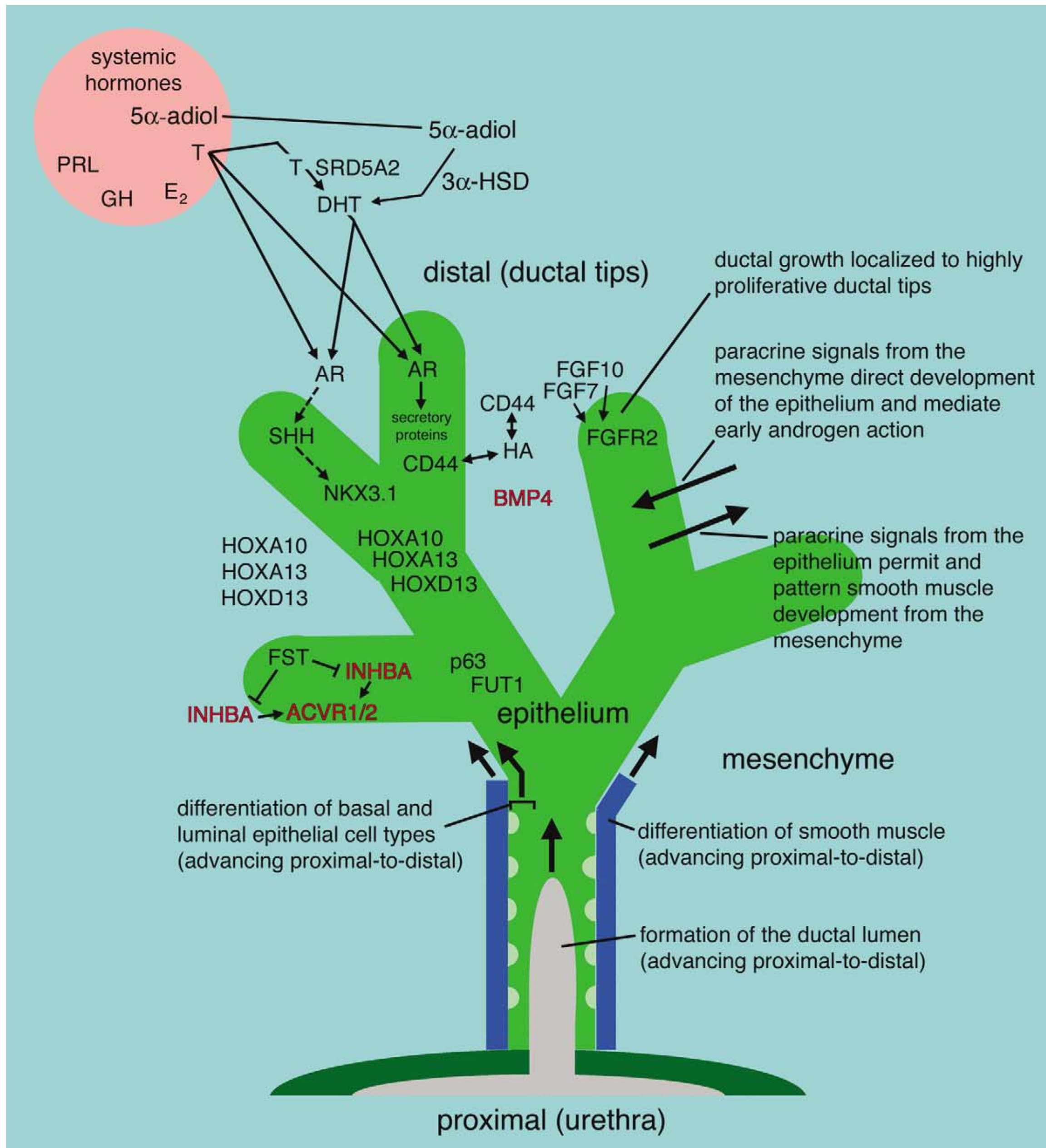

Fig. 2. Features of prostatic branching morphogenesis. The diagram shows a generic network of developing prostatic ducts. The stage of development, with solid undifferentiated epithelial cords in the distal region and a forming lumen and cellular differentiation in the proximal region, reflects the early postnatal period in rodent prostate development. The developing ductal epithelium is shown in green; the developing prostatic mesenchyme is shown in blue; and the forming ductal lumen is shown in pink. Text annotations indicate general features of prostatic development that have been uncovered by descriptive and experimental embryological studies. Hormones and gene products that have been implicated in prostate development are also shown with developmental expression in epithelium or mesenchyme indicated by the location(s) of the gene or hormone symbol. Factors that act positively to promote growth or morphogenesis of the prostate are shown in black text. Factors that act negatively to limit growth or morphogenesis are shown in red text.

luminal and basal cell cytokeratins) differentiates into the distinct luminal and basal cell lineages each expressing their characteristic subset of cytokeratins. At the same time that the prostatic epithelium differentiates into distinct basal and luminal epithelial cell types, the prostatic mesenchyme/stroma differentiates into a layer of smooth muscle that surrounds the prostatic ducts (Hayward et al., 1996b).

Branching morphogenesis is almost entirely complete by 2 weeks after birth in the mouse (Sugimura et al., 1986a). Serum testosterone (T) levels are low during this time, and the increase in prostatic wet weight is modest. At puberty, serum T levels rise significantly, and prostatic wet weight and DNA content increase more rapidly than during the early postnatal period (Donjacour and Cunha, 1988). Functional cytodifferentiation of luminal epithelial cells occurs with the expression of the prostate-specific secretory pro-

teins. In mice, prostatic secretory proteins are first detectable just prior to puberty (about postnatal day 20) and greatly increase in abundance as serum T levels rise (Donjacour et al., 1990; Mills et al., 1987c). At least two distinct sets of secretory proteins are expressed by different regions of the mouse prostate. One group of proteins is secreted by the VP, and the other by the DLP and AP (Donjacour et al., 1990; Mills et al., 1987a, b, c). In the human prostate, some secretory activity is detectable during fetal life (Xia et al., 1990) presumably due to the action of fetal testicular androgens.

Morphological and cellular structure of the adult prostate

At sexual maturity, the mouse prostate is a multilobed gland (Fig. 1B) arranged around the urethra at the base of the bladder. Due to lobe-specific differences in the patterns of branching morphogenesis, the final shape of each lobe is distinct. In addition, the lobes have distinct histologic features with extensive epithelial-infolding in the AP, significant but less extensive epithelial-infolding in the DLP, and minimal epithelial-infolding in the VP (Fig. 1B, inset). Regional differences in cell morphology, rates of DNA synthesis, and secretory activity are also observed along the proximal–distal (urethra to ductal tip) axis of prostatic ducts (Lee et al., 1990; Sugimura et al., 1986b). The human prostate has a more compact adult morphology without distinct lobes. It is roughly the size and shape of a walnut (20 g and 4×2.5 cm). The organization of the adult human prostate is commonly described in terms of three zones: a central zone, a transition zone, and a peripheral zone, reflecting three distinct sets of ducts present in the human prostate (McNeal, 1983). Comparative observations of prostatic development in rodents and humans demonstrate that morphogenesis occurs in an analogous manner in both humans and rodents with several distinct sets of epithelial buds growing out of the urethra into the UGM (Timms et al., 1994b). Nevertheless, compelling molecular evidence for homology between specific rodent prostatic lobes and human prostatic zones has yet to be identified.

Mature prostatic ducts contain three major cell types, luminal secretory epithelial cells, basal epithelial cells, and stromal smooth muscle cells, that can be distinguished by their patterns of differentiation marker expression (Fig. 1C). Other less common cell types include neuroendocrine cells as well as rare basal epithelial cells with unique marker expression profiles that are candidates for epithelial stem cells (Fig. 1C; Collins et al., 2001; Wang et al., 2001). The relative distribution of cell types is somewhat different between mouse and human prostates. In both species, the ductal lumen is lined by tall columnar secretory epithelial cells. These cells have an apical–basal polarity and secrete prostatic proteins and fluids from their apical surface into the prostatic lumen. In humans, basal epithelial cells form a nearly continuous layer between the secretory cells and the basement membrane, while in mice, fewer basal cells are present such that they are dispersed as a discontinuous layer around the ducts. Neuroendocrine cells are present as rare cells within the epithelial layer of both developing and adult human prostate. While basal and luminal epithelial cells are derived from the epithelium of the cloaca, the origin of neuroendocrine cells is controversial. One hypothesis holds that neuroendocrine cells share a common progenitor with secretory cells (Xue et al., 1998). The other hypothesis posits that neuroendocrine cells are of neural crest origin, migrating to the UGE through the UGM from the paraganglia early in prostatic development (Aumuller et al., 1999). Neuroendocrine cells have not been extensively studied in rodents. Rats may not have neuroendocrine cells (Angelsen et al., 1997), while murine neuroendocrine cells have only recently been identified in the adult prostate (Garabedian et al., 1998). Recent research with both mouse and human prostates has also identified a rare subset of basal cells that are candidates to be epithelial stem cells (Fig. 1C; Collins et al., 2001; Wang et al., 2001). In mice, rare basal cells have been identified with a marker expression profile more characteristic of undifferentiated UGE than the other adult epithelial cell types (Wang et al., 2001). In humans, rare basal cells have been identified with high expression of $\alpha 2\beta 1$-integrin, a marker expressed by putative stem cells in other tissues (Collins et al., 2001). It is not yet clear if the cell types described in these two studies are identical.

The stromal layer of both the mouse and the human prostate is largely composed of smooth muscle. This layer is much thicker in the human prostate such that the human prostate has a higher ratio of stromal to epithelial cells than the mouse. Although mostly smooth muscle, the stromal layer also contains fibroblastic, neuronal, lymphatic, and vascular cell types.

Mechanisms of prostatic development

Research into the mechanisms that underlie prostatic development has been facilitated by the use of experimental embryological techniques including tissue recombination and grafting experiments and in vitro organ cultures as well as by study of mice and humans harboring spontaneous or engineered mutations. This work has provided insight into the roles of steroid hormones, cell–cell communication, and specific gene products in prostatic development.

Roles of steroid hormones in prostatic development

Systemic androgens produced by the fetal testis act to specify development of the UGS into prostate. In the absence of androgens, as in females, the UGS forms the lower portion of the vagina as well as the urethra. Cellular response to systemic androgens is mediated by nuclear androgen receptors that are activated by testosterone (T) or dihydrotestosterone (DHT). The requirement for and sufficiency of androgens in establishing prostate identity in the

UGS is shown by the absence of a prostate in mice or humans that lack functional androgen receptors due to inactivating mutations (Brown et al., 1988; Charest et al., 1991; Gaspar et al., 1991; He et al., 1991; Lubahn et al., 1989), and by the development of a prostate in female urogenital sinuses exposed to androgens (Takeda et al., 1986). Synthesis of T by the fetal testis begins between 13 and 14 dpc in mice (Pointis et al., 1979, 1980) and around 9 weeks in humans (Siiteri and Wilson, 1974). Testosterone in fetal circulation is significantly greater in male than female mice (Pointis et al., 1979, 1980). In the UGS, T could activate androgen receptors through direct binding to the androgen receptor and through local conversion of circulating T to the more potent androgen receptor agonist, DHT, by the enzyme Δ^4-3- ketosteroid-5α-reductase (5α-reductase) type 2 (Russell and Wilson, 1994). DHT has a 10-fold greater affinity for the androgen receptor than T (Deslypere et al., 1992). When conversion of T to DHT is blocked in the prostate by mutations in the gene encoding 5α-reductase type 2, the UGS is specified as prostate but prostatic growth and development are greatly reduced (Andersson et al., 1991; Mahendroo et al., 2001). The fact that blocking conversion of T to DHT in the developing prostate causes a profound reduction in organ growth and morphogenesis argues that systemic T (and its subsequent conversion to DHT) is an important mediator of testicular androgen synthesis during prostatic development in mice and humans. The prostatic developmental defects in mice and humans lacking 5α-reductase type 2 further demonstrate that DHT is a crucial local mediator of testicular androgen action.

Recently, an investigation of prostate specification in marsupials has implicated a second testicular androgen, 5α-androstane-3α, 17β-diol (5α-adiol), as the circulating androgen that acts to specify the UGS as prostate (Shaw et al., 2000). In contrast to the situation in rodents, serum T levels were not found to be sexually dimorphic in the marsupial, *Macropus eugenii*; however, serum 5α-adiol levels were found to be higher in males than in females at the time of prostate specification. In addition, 5α-adiol treatment caused female urogenital sinuses to initiate prostatic development, appearing to act via local conversion of 5α-adiol to DHT by 3α-hydroxysteroid dehydrogenase. In the developing rat prostate, 5α-adiol has the potential to stimulate branching morphogenesis in vitro with almost the same potency as T and DHT (Foster and Cunha, 1999), but its efficacy at stimulating initial prostatic budding remains untested. Further work is needed to determine whether 5α-adiol acts together with T as a systemic mediator of testicular androgen synthesis during prostatic development in mice and humans.

The first known response to androgens by the murine UGS is the expression of the homeobox gene *Nkx3.1* in patches of urogenital sinus epithelium (UGE) at 15.5 dpc, which is 2 days before the appearance of prostatic buds (Sciavolino et al., 1997). The first morphological response of the murine UGS to androgens is the growth of prostatic epithelial buds from the UGE into the UGM at around 17.5 dpc. In addition to these early roles in establishing prostatic identify, androgens are important for stimulating ductal growth and branching morphogenesis as well as for establishing functional differentiation of luminal secretory epithelial cells. Tissue recombination and grafting experiments utilizing urogenital sinuses from Tfm mice that lack androgen receptors have shown that mesenchymal receptors are required for establishing prostate identity (Bhatia-Gaur et al., 1999; Cunha and Lung, 1978) and for stimulating ductal morphogenesis. Epithelial receptors are required for establishing secretory function in the epithelium (Donjacour and Cunha, 1993). The fact that mesenchymal but not epithelial androgen receptors are required for epithelial branching morphogenesis demonstrates that paracrine signals from the UGM mediate the action of androgens on the UGE during prostatic development.

In addition to androgens, prostatic development is very sensitive to levels of estrogenic compounds. Male embryos that develop between two females (relative to males that develop between two males) have higher serum estradiol, lower serum testosterone, increased expression of the androgen receptor in the prostate, and larger prostates in adulthood that arise from more extensive developmental growth (Timms et al., 1999). Similar prostatic enlargement is observed when male fetuses are exposed to estrogenic compounds at extremely low environmental levels (Nagel et al., 1999; vom Saal et al., 1997). In contrast, developmental exposure of males to high levels of estrogenic compounds causes reduced prostatic growth and altered glandular architecture (Prins, 1992). Genetic manipulations of the estrogen synthesis pathway in mice that lead to excess or reduced estrogen levels also affect prostatic size and differentiation (Jarred et al., 2002). It should be noted that, in each of the cases where changes in estrogen levels were observed to alter prostatic development, the changes were systemic and led to complex systemic changes in the overall endocrine environment. Mice with inactivating mutations in the known nuclear estrogen receptors, estrogen receptor α or estrogen receptor β, as well as α, β compound mutant mice have normal prostatic development (Jarred et al., 2002). Together, these data suggest that prostatic development is sensitive to the overall endocrine environment, including estrogen levels, but local action of the known nuclear estrogen receptors is not required for prostatic development in the context of a normal endocrine environment.

Epithelial–mesenchymal interactions in prostatic development

Organogenesis of the prostate is dependent on mesenchymal–epithelial interactions such that morphogenesis and differentiation of both the epithelium and mesenchyme are abortive if the epithelium and mesenchyme are grown sep-

arately. The previously mentioned grafting and tissue recombination experiments with Tfm mice make clear that the early developmental effects of androgens on the UGE are mediated by paracrine signals from the UGM. Additional grafting and tissue recombination experiments have demonstrated that paracrine signals from the developing UGM also direct lobe-specific identity in the juxtaposed epithelium.

Complete UGM as well as the ventral subdivision of the UGM have been recombined and grafted with a variety of partner epithelia. Ventral UGM partnered with complete (dorsal + ventral) UGE forms prostatic ducts that express secretory proteins characteristic of ventral prostate (Timms et al., 1995), while complete UGM partnered with adult ventral epithelium forms prostatic ducts that express secretory proteins characteristic of anterior and dorsolateral prostate as well as ventral prostate (Hayashi et al., 1993). UGM partnered with embryonic or adult bladder epithelium also forms prostatic ducts. Grafts in which UGM is partnered with epithelia from other anatomical locations, including the seminal vesicle, salivary gland, and esophagus, form tissues with differentiated epithelia characteristic of the anatomical site of origin of the epithelium (Cunha et al., 1987). These experiments reveal several characteristics of paracrine signals from the UGM and the competence of epithelia to respond. First, there are regionally restricted domains within the UGM that specify a dorsal or ventral fate for the prostatic epithelium. Second, other epithelial derivatives of the cloaca (e.g., bladder) can be induced to form prostate by UGM, but epithelia from other locations cannot. Third, the potential of heterotypic epithelia to respond to UGM by forming prostatic ducts extends into adulthood. Together, these results suggest that development of the prostate is spatially restricted by limited domains of prostate-inducing paracrine signals in the mesenchyme of the developing male urogenital tract. Finally, epithelial potential to respond to paracrine signals from the UGM by forming prostate is restricted to endodermal epithelia with similar embryonic origin to the prostate. Interestingly, although the morphological form of the prostate varies significantly among mammals, the instructive signals from the UGM are conserved across species lines. This has been demonstrated by heterospecific tissue recombination and grafting experiments utilizing tissues from human, mouse, rabbit, and rat (Aboseif et al., 1999; Cunha et al., 1983).

Additional grafting and tissue recombination experiments have demonstrated that interactions between the developing prostatic mesenchyme and epithelium are reciprocal. Rat UGM has been grafted alone or in combination with either mouse or human prostatic epithelia. Grafts of UGM alone formed little smooth muscle. Grafts that included either mouse or human prostatic epithelia formed a smooth muscle layer around the prostatic ducts that developed in the grafts. However, grafts of UGM paired with mouse epithelium formed prostatic ducts surrounded by a thin smooth muscle layer, i.e., with the mouse stromal architecture. Conversely, grafts of UGM paired with human epithelium formed ducts surrounded by a thick smooth muscle layer, i.e., with the human stromal architecture (Hayward et al., 1998). In addition, grafts with human epithelium form a continuous layer of basal epithelial cells beneath the luminal secretory epithelial cells, thus recapitulating the prostatic epithelial architecture found in humans. These experiments demonstrate that instructive paracrine signals from the prostatic epithelium induce and pattern the surrounding smooth muscle. They also reveal the presence of intrinsic mechanisms within the epithelium that establish the relative distribution of basal and luminal epithelial cells.

Molecular control of prostate development

Several genes and gene families have been suggested to play a role in prostatic development. The evidence for assigning a developmental role to a particular gene varies from weak evidence such as descriptive work that shows that a particular gene is expressed during prostatic development to compelling evidence such as a specific prostatic developmental phenotype caused by inactivating mutations in the gene. For the purposes of this review, only genes implicated in prostatic development by work that includes functional studies on intact animals or functional studies utilizing primary developing organs, tissues, or cells will be presented.

The phenotypes of both spontaneous and engineered mutations in mice and humans have provided direct evidence for the roles of several genes in prostatic development (summarized in Table 1). In a few cases, experimental evidence suggests that some of these genes act in redundant or serial fashion. The homeobox containing transcription factors HOXA13 and HOXD13 are both required for normal morphogenesis of the prostate and act in a partially redundant fashion. *Hoxa13*$^{+/-}$ mice have small prostates with reduced branching morphogenesis of the VP and DLP (Podlasek et al., 1999b). *Hoxd13*$^{-/-}$ mice also have a smaller VP and DLP with reduced DLP branching morphogenesis (Podlasek et al., 1997). Compound *Hoxa13*$^{+/-}$:*Hoxd13*$^{-/-}$ mutant mice have a more severe failure of prostate development, including the absence of the AP (Warot et al., 1997). The enzyme 5-α reductase type 2 (encoded by the *Srd5a2* gene) synthesizes the potent androgen receptor agonist, DHT, from circulating T (Russell and Wilson, 1994). Similarly, the enzyme 3α-hydroxysteroid dehydrogenase (3α-HSD, encoded by the *Akr1c4* gene) synthesizes DHT from circulating 5α-adiol. Thus, *Srd5a2* and *Akr1c4* function upstream of the androgen receptor, which in turn up-regulates the *Shh* and *Nkx3.1* genes either directly or indirectly (Podlasek et al., 1999a; Sciavolino et al., 1997). Furthermore, analysis of *Shh*$^{-/-}$ mice places SHH upstream of the NKX3.1 transcription factor in prostate development since the UGS of *Shh*$^{-/-}$ mice fails to express *Nkx3.1* transcripts (Schneider et al., 2000).

Additional genes have been implicated in prostate development by functional studies based on the manipulation of

Table 1: Phenotypes of spontaneous and engineered mutations affecting prostatic development

Symbol	Gene	Protein type	Developmental expression	Alleles[1]	Prostate phenotype[1]	Comments	References
Ar	*androgen receptor*	steroid receptor	mesenchyme + epithelium	Tfm (mouse) AIS (human)	no prostate	mesenchymal receptors are required to specify organ identity and promote morphogenesis; epithelial receptors are required for secretory differentiation	Brown *et al.*, 1988; Luban *et al.*, 1989; He *et al.*, 1991; Charest *et al.*, 1991; Gaspar *et al.*, 1991
Bmp4	*bone morphogenetic protein 4*	secreted	mesenchyme	mouse +/- knockout	increased branching morphogenesis in AP and VP	recombinant protein reduces prostatic epithelial proliferation *in vitro*	Lamm *et al.*, 2001
Ghr	*growth hormone receptor*	transmembrane receptor		transgenic antagonist	small prostate with reduced branching		Ruan *et al.*, 1999
Hoxa10	*homeo box A10*	transcription factor	mesenchyme +epithelium	mouse -/- knockout	reduced AP branching, partial AP to DLP transformation		Podlasek *et al.*, 1999
Hoxa13	*homeo box A13*	transcription factor	mesenchyme +epithelium	Hd +/- (mouse)	small size and reduced branching in VP and DLP	more profound defects in prostate morphogenesis are observed in *Hoxa13 +/-:Hoxd13-/-* mice	Podlasek *et al.*, 1999; Warot *et al.*, 1997
Hoxd13	*homeo box D13*	transcription factor	mesenchyme +epithelium	mouse -/- knockout	small size of VP and DLP, reduced DLP branching	more profound defects in prostate morphogenesis are observed in *Hoxa13 +/-:Hoxd13-/-* mice	Podlasek *et al.*, 1997; Warot *et al.*, 1997
Igf1	*insulin-like growth factor 1*	secreted		mouse -/- knockout	small prostate with reduced branching	*Igf1-/-* mice have dramatically lower circulating androgen levels so some observed phenotypes may be indirect	Baker *et al.*, 1996; Ruan *et al.*, 1999
Nkx3.1	*NK-3 transcription factor, locus 1*	transcription factor	epithelium	mouse -/- knockout	reduced branching and altered secretory protein expression profile	earliest known molecular marker of the prostatic epithelium, androgens up-regulate expression of *Nkx3.1*	Bhatia-Gaur *et al.*, 1999; Schneider *et al.*, 2000; Tanaka *et al.*, 2000
p63	*tumor protein p63*	nuclear	epithelium	mouse -/- knockout	no prostatic epithelial buds present at birth	*p63-/-* mice die at birth so postnatal developmental phenotypes are not known; p63 is expressed by basal epithelial cells in the adult prostate	Signoretti *et al.*, 2000
Prl	*prolactin*	secreted	circulating hormone	mouse -/- knockout	small VP		Steger *et al.*, 1998
Shh	*sonic hedgehog*	secreted	epithelium	mouse -/- knockout	loss of *Nkx3.1* expression in the urogenital sinus	inhibitory antibodies against SHH protein inhibit prostate morphogenesis, androgens up-regulate expression of *Shh; Shh-/-* mice die before prostate development occurs	Podlasek *et al.*, 1999; Schneider *et al.*, 2000
Srd5a2	*steroid 5-alpha reductase 2*	enzyme	mesenchyme	mouse -/- knockout PPSH (human)	small prostate		Andersson *et al.*, 1991; Mahendroo *et al.*, 2001

[1] Abbreviations: Tfm, testicular feminization; AIS, androgen insensitivity syndrome; Hd, hypodactyly; PPSH, pseudovaginal perineoscrotal hypospadias; AP, anterior prostate; DLP, dorsolateral prostate; VP, ventral prostate

prostatic organ cultures to augment descriptive work. Two members of the fibroblast growth factor (FGF) family of secreted proteins, FGF7 and FGF10, are expressed in the mesenchyme of the developing prostate. These growth factors are thought to act through FGFR2 expressed by the prostatic epithelium. Furthermore, work with prostate organ cultures and isolated prostatic cells suggests that these factors act to promote prostatic ductal morphogenesis by promoting epithelial proliferation (Sugimura et al., 1996; Thomson and Cunha, 1999). Analysis of the developmental expression patterns and effects of recombinant proteins on in vitro organ cultures have also implicated the secreted factors activin A and its binding protein follistatin in regulating prostatic morphogenesis (Cancilla et al., 2001). Activin A is expressed in both the developing prostatic mesenchyme and epithelium; its receptors are expressed in the epithelium. Follistatin, an activin A antagonist, is also expressed in the epithelium. Recombinant forms of these proteins have opposing effects with activin A acting to limit, and follistatin acting to promote epithelial growth and morphogenesis. Hyaluronan, a polysaccharide component of the extracellular matrix, and its cellular receptor, CD44, have also been implicated in prostatic morphogenesis. Hyaluronan is present in the mesenchymal matrix of the developing prostate, and CD44 is expressed by both mesenchymal and epithelial cells. Treatment of prostatic organ cultures with agents that antagonize hyaluron/CD44 function including hyaluronan hexasaccharides, hyaluronidase, and anti-CD44 antibodies all impaired prostatic morphogenesis (Gakunga et al., 1997). Fucosyltransferase 1, a transmembrane enzyme present in the secretory pathway and at the cell surface, is expressed by the developing prostatic epithelium and plays a role in prostatic development. Inhibitory antibodies directed against fucosyltransferase 1 reduce epithelial proliferation of prostatic organ cultures (Marker et al., 2001). Interaction between urokinase plasminogen activator and the membrane urokinase plasminogen activator receptor also appears to play a pro-differentiation/pro-survival role during prostatic development as peptides that disrupt this interaction inhibit growth and differentiation of prostatic organ cultures with associated increases in apoptosis (Elfman et al., 2001).

Major unresolved questions

Many years of descriptive and experimental embryological work have provided substantial insight into the roles of circulating hormones and epithelial–mesenchymal interactions in controlling development of the prostate (Fig. 2). In recent years, several specific gene products and genetic pathways have been identified that act at the molecular level to control some aspects of prostatic development (Table 1 and Fig. 2). Nevertheless, these genes do not explain in molecular terms the complex biological interactions that descriptive and experimental embryological studies have elucidated in the control of prostatic development.

One major unresolved question is the nature of the mesenchymal factors that mediate the actions of androgens on the developing prostatic epithelium. Tissue recombination and grafting experiments with Tfm mice have demonstrated that epithelial budding, branching morphogenesis, and early epithelial differentiation are dependent on mesenchymal androgen receptors. Consequently, there must be androgen-dependent paracrine cues from the mesenchyme that direct epithelial morphogenesis. One current hypothesis holds that androgen-dependent differences in the differentiation of smooth muscle within the UGM may play a role in this paracrine interaction by creating a physical barrier between the epithelium and constitutive mesenchymal growth factors in females but not in males (Thomson et al., 2002). However, the causal relationship between more extensive smooth muscle differentiation in the female UGM and the lack of epithelial budding in the female UGE has not yet been established.

A second major unresolved question is the nature of factors that control region-specific identity in the prostate. The different prostatic lobes have different overall shapes and patterns of branching morphogenesis. They also have distinct histologic appearances and distinct patterns of secretory protein production. Grafting and tissue recombination experiments have demonstrated that region specific identity in the epithelium is established, at least in part, by paracrine cues from the mesenchyme. Thus, there must regulatory genes with region-specific expression in both the prostatic mesenchyme and epithelium as well as region specific paracrine cues from the mesenchyme to the epithelium. Currently, the only gene that has been specifically implicated in controlling an aspect of region-specific identity is *Hoxa10* that is expressed in both the mesenchyme and epithelium of the developing prostate. Mutations in this gene have been reported to cause a partial transformation of AP to DLP based on ductal morphology and branching pattern (Podlasek et al., 1999c). However, this proposed transformation was incomplete and only partially penetrant.

An important issue related to region-specific identity within the prostate is whether homology between particular prostatic lobes in rodents and the prostatic zones in humans can be established. This issue has direct relevance for understanding and modeling human prostatic diseases because prostatic diseases occur in a highly region-specific manner in humans with prostatic adenocarcinoma predominantly a disease of the peripheral zone and benign prostatic hyperplasia predominantly a disease of the transition zone. Comparative anatomical studies of developing and adult human and rodent prostates have not provided a clear resolution to this question. The identification and characterization in mice and humans of the molecular factors that control region-specific prostate identity is needed.

Finally, descriptive and experimental embryological studies have shown that there are differences in cellular

proliferation, secretory activity, and cellular architecture along the proximal–distal axis of the prostatic ducts as well as paracrine cues from the epithelium that direct mesenchymal development. None of these features of prostate development can currently be explained in molecular terms.

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