

Effect of Mesenchymal Glandular Inductors on the Growth and Cytodifferentiation of Neonatal Mouse Seminal Vesicle Epithelium

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ABSTRACT: To investigate the developmental properties of glandular mesenchymal inductors along the cranial-caudal extent of the developing male urogenital tract, neonatal mouse seminal vesicle epithelium (SVE) was combined with mesenchyme of the seminal vesicle (SVM), urogenital sinus (UGM), bulbourethral gland (BUG-M), or bladder (BLM) and grafted under the renal capsule of adult syngeneic or athymic male mice. Both SVM + SVE and UGM + SVE tissue recombinants expressed SV histogenesis and SV secretory proteins. BUG-M + SVE recombinants exhibited extensive growth as evidenced by a 36-fold increase in wet weight and a 27-fold increase in DNA content; however, the glandular structures that were induced in the SVE lacked the convoluted mucosa typical of

SV. Furthermore, neither SV nor prostatic secretory proteins were detected in these recombinants. SVE grown in association with BLM failed to develop altogether. Thus, the ability to promote SV histogenesis and function is distinctly different in mesenchyme of cranial (SVM and UGM) versus caudal (BUG-M) regions. This implies the existence of a glandular inductive field in the developing male urogenital tract within which inductive activity varies regionally.

Key words: Epithelial-mesenchymal interaction, seminal vesicle epithelium, bulbourethral gland mesenchyme, bladder mesenchyme, prostatic development, ductal branching morphogenesis.

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The role of mesenchyme in androgen-dependent epithelial growth and cytodifferentiation in the urogenital tract has been well documented (Cunha et al, 1983a, 1987, 1992). Prostatic development can be induced by several types of mesenchyme, e.g., the urogenital sinus (UGM, fetal prostatic mesenchyme) (Cunha, 1972; Cunha et al, 1983b; Takeda et al, 1990; Hayashi et al, 1993), seminal vesicle (SVM) (Cunha, 1972; Kimbara et al, unpublished data), bulbourethral gland (BUG-M) (Kimbara et al, unpublished data), and vagina, i.e., female UGM (Cunha, 1975). This diverse group of prostatic mesenchymal inductors implies the existence of common inductive activity shared by all of the above mesenchymes. One universal feature of all prostatic inducers is the expression of androgen receptors (AR), which appear to be absolutely essential for androgen-dependent prostatic development (Cooke et al, 1991; Cunha et al, 1980b, 1987;

Shannon and Cunha, 1983). This conclusion has been drawn from analysis of tissue recombinants constructed with epithelium and mesenchyme from normal and Tfm (testicular feminization) mice. Tfm mice are completely androgen resistant due to a mutated, nonfunctional AR (French et al, 1990; He et al, 1991). In such experiments, prostatic development occurs in the presence of androgens when normal UGM is grown in association with either normal or Tfm epithelium. Conversely, prostatic tissue never develops when mesenchyme from Tfm mice is utilized regardless of the genotype of the epithelium (Cunha and Lung, 1978; Cunha et al, 1980a; Lasnitzki and Mizuno, 1980). The requirement of AR in all prostatic mesenchymal inductors emphasizes the paracrine nature of androgen action and the importance of mesenchyme as the primary androgen target tissue, a concept also supported by comparable studies in the developing mammary gland (Kratochwil and Schwartz, 1976; Drews and Drews, 1977). While several different mesenchymes can induce prostatic development, each also expresses additional tissue-specific inductive properties. For example, SVM, which is physically contiguous with UGM, can induce prostatic development from endodermal urogenital sinus-derived epithelia but induces seminal vesicle (SV) differentiation from a variety of embryonic and adult epithelial derivatives of the mesodermal Wolffian duct (Cunha, 1972;

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Higgins et al, 1989a,b; Turner et al, unpublished data; Cunha et al, 1991). Likewise, UGM can induce prostatic development in a variety of endodermal epithelia (Boutin et al, 1991) but induces SV development from Wolffian duct-derived epithelia (Cunha, 1972; Cunha et al, unpublished data). BUG-M, while capable of inducing prostatic growth and morphogenesis (Hayashi et al, 1993; Kimbara et al, unpublished data), is located caudal (Fig. 1) to the developing prostate (Cunha and Lung, 1979). BUG-M normally induces BUG differentiation from the urogenital sinus below the prostate. In this paper, using seminal vesicle epithelium (SVE) as the test subject, SVM, UGM, BUG-M, and bladder mesenchyme (BLM) were employed in order to examine regional differences in the inductive properties of male urogenital gland inductors. Because distinctive differences in branching morphogenesis, cytodifferentiation, and secretory proteins exist between the prostate, BUG, and SV (Higgins and Parker, 1980; Chen et al, 1987; Cooke et al, 1987a,b; Cunha et al, 1987; Fawell et al, 1987; Curry and Atherton, 1990; Shima et al, 1990), regional differences in the glandular inductors within the male genital tract were assessed in both morphological and functional terms.

Materials and Methods

Animals and Tissue Recombination

The rudiments of SVs, bulbourethral glands (BUGs), and bladders (BLs) were excised from newborn (0-day-old) BALB/c male mice or Sprague-Dawley rats obtained from Simonsen laboratories (Hollister, California) or the Cancer Research Laboratory at the University of California (Berkeley, California) (Fig. 1). Urogenital sinuses (UGS) and SVs were excised from 18-day-old embryonic Sprague-Dawley rats. BLs were cut at the bladder neck with fine forceps as described previously (Cooke et al, 1987b; Shima et al, 1990) and chopped into several pieces (1.5–2-mm squares) about two-fold larger than the size of a single SV rudiment. SVs, BUGs, UGS, and BL pieces were incubated at 4°C for 90 minutes with Hank's balanced salt solution (Ca^{2+} and Mg^{2+} free) containing 1% trypsin, and SVE, BUG-M, UGM, SVM, and BLM were isolated using an iris scalpel and fine forceps. SVE isolated from a single SV was recombined with 1 or 3 BUG mesenchymes or with a single SVM, UGM, or BLM, as described previously (Cunha et al, 1983c; Higgins et al, 1989b). It should be noted that with experience the UGS, SV, and BUG rudiments can be trimmed to uniform sizes. Tissue recombinants (SVM + SVE, UGM + SVE, BLM + SVE, or BUG-M + SVE) were prepared as described previously and grafted under the renal capsules of adult syngeneic or athymic male mice anesthetized with Avertin (tribromoethanol). In some cases chimeric tissue recombinants were prepared, i.e., rat UGM + mouse SVE and rat SVM + mouse SVE. As a control, isolated mesenchyme was grafted by itself to assess possible contamination with residual epithelium (Table 1).

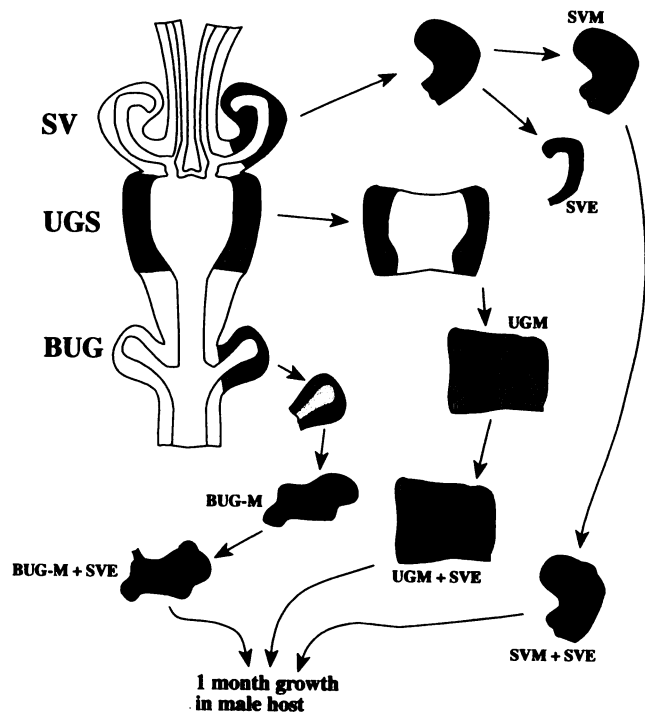


FIG. 1. Anatomical organization of the male urogenital tract showing the cranial-caudal organization of glands. Tissue recombinants were prepared as indicated. Not shown is the BLM + SVE tissue recombinant. Abbreviations as per text.

One to four weeks after grafting, host mice were killed by cervical dislocation, and the tissue recombinants were harvested from the renal capsule. Some recombinants were weighed with a precision balance, dissolved in 0.5 N NaOH (2–4 hours at 60°C), and neutralized with an equal volume of 0.5 N HCl for the measurement of DNA content using a Hoechst dye fluoro-

Table 1. Developmental response of SVE grown in association with SVM, UGM, BUG-M, or BLM

Tissues grafted*	No. of grafts analyzed	Ductal morphology	SV morphology	SV secretion	Percent response†
BUG-M‡	25		N/A	N/A	N/A
BLM‡	15		N/A	N/A	N/A
SVM‡	25		N/A	N/A	N/A
UGM‡	10		N/A	N/A	N/A
SVM + SVE	20	+	+	+	100
UGM + SVE	9		+	+	100
BUG-M + SVE	75	+	–	–	100
BLM + SVE	45	–	–	–	0

* SVM, seminal vesicle mesenchyme; UGM, urogenital sinus mesenchyme; BLM, bladder mesenchyme; BUG-M, bulbourethral gland mesenchyme.

† After 4 weeks of growth, each specimen was judged by wholemount photography, histology, or immunocytochemistry.

‡ Of 75 control grafts of mesenchyme, only a single BUG-M contained epithelial tissue. All remaining grafts (74/75) were recovered as undifferentiated fibromuscular tissues.

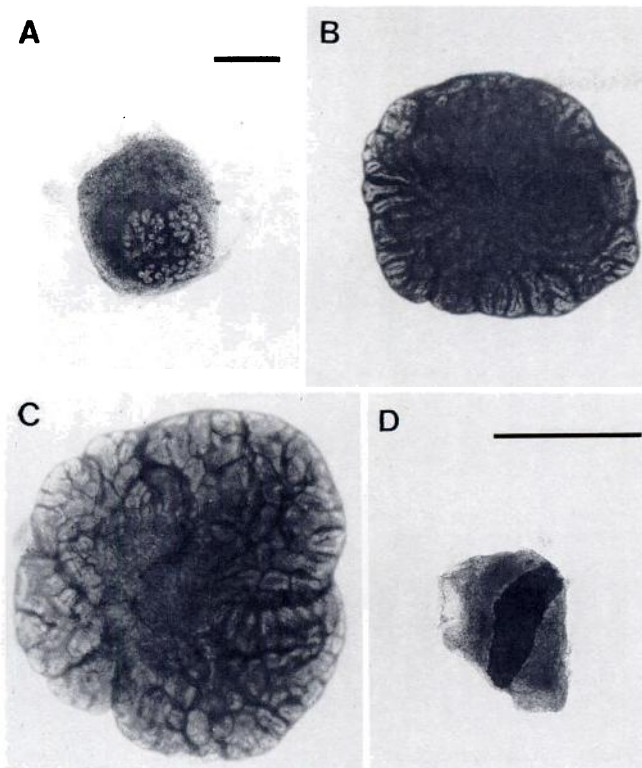


FIG. 2. Wholemount pictures of tissue recombinants. A, B, and C: BUG-M + SVE recombinants prepared with a single BUG-M, 1 (A), 3 (B), and 4 weeks (C) after grafting underneath the renal capsule of adult male mice ($\times 25$). BLM + SVE recombinant (D) 4 weeks after *in vivo* grafting ($\times 60$). Note branching morphogenesis of SVE increasing according to the period of growth in Figure 1A–C, and the degenerated epithelium on the BLM in Figure 1D. A, B, and C are the same magnification. Bar at the right upper corner of A and D is 400 μm .

metric assay (Labarca and Paigen, 1980). The values were expressed as mean \pm SE.

Some specimens were fixed in phosphate-buffered saline (PBS) containing 4% *p*-formaldehyde at room temperature (RT) for histological analysis. Others were used for collection of secreted proteins, which were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), as indicated below.

Histology and Immunocytochemistry

Paraformaldehyde-fixed tissues were washed in fresh PBS, dehydrated in graded ethanol solutions, cleared in toluene, and embedded in paraffin. Sections were cut at 5 μm and air dried onto poly-L-lysine-coated slides. Some sections were stained with hematoxylin and eosin. Others were used for immunocytochemistry to identify mouse SV secretory proteins with a polyclonal rabbit antibody (anti-mouse SVS, 1/500 dilution), as described previously (Higgins et al, 1989a). Briefly, the sections were treated successively at room temperature with PBS containing 1% BSA and 1.5% horse serum (Vector Labs, Burlingame, California), anti-mouse SVS (1/500 dilution), biotinylated donkey anti-rabbit IgG (1/100 dilution, Amersham Corp, Arlington Heights, Illinois), and avidin-biotinylated horseradish peroxidase complex (ABC reagent, Vector Labs, Burlingame, California). The

color was developed using diaminobenzidine and H_2O_2 in the presence of CoCl_2 (Higgins et al, 1989a).

Polyacrylamide Gel Electrophoresis

Proteins secreted from adult mouse SVs and BUGs as well as from SVM + SVE, UGM + SVE, BLM + SVE, and BUG-M + SVE tissue recombinants grown for 4 weeks in male hosts were collected by slicing open the glandular lumina with a scalpel and gently squeezing the secretion from the tissues into Ca^{2+} - and Mg^{2+} -free PBS containing 0.02 M EDTA and protease inhibitors (1 mM phenylmethylsulfonylfluoride, 1 mM iodoacetic acid, 1 mM *N*-ethylmaleimide, 0.1 mM leupeptin, and 0.1 mM pepstatin-A [all from Sigma, St. Louis, Missouri]). The collected luminal secretions were mixed with an equal volume of 10% SDS and boiled for 5 minutes. Reduced proteins (20–30 $\mu\text{g}/\text{lane}$) were separated by electrophoresis in polyacrylamide (10–20% linear gradients) slab gels using the discontinuous buffer system of Laemmli (1970) with 0.1% SDS as described earlier (Brooks and Higgins, 1980) and visualized by staining with Coomassie blue (Sigma).

Western Blots

Proteins were collected as above and separated on 10–15% SDS gradient gels using the PhastGel system (2–3 μg protein/lane, Pharmacia). Proteins were transferred to nitrocellulose and stained with Indian ink (1/1,000 dilution in PBS/0.1% Tween) for 1 hour. Blots were blocked in 5% bovine serum albumin (BSA)/PBS for 1 hour at 37°C and then incubated for 1 hour at RT in primary antibody. Following an overnight wash in PBS/0.1% Tween at 4°C, blots were incubated at RT for 1 hour in a 1/50,000 dilution of horseradish peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad) in 1% BSA/PBS, and washed for an additional 2 hours. Bands were visualized using the ECL western blotting detection reagents (Amersham). Primary antibodies were diluted 1/8,000 in 5% BSA/PBS for anti-mouse SVS (Higgins et al, 1989a) and 1/5,000 in 1% BSA/PBS for anti-MP-3 (Mills et al, 1987) and anti-mouse DLP1 (Donjacour et al, 1990). All blots contained appropriate positive and negative controls.

Results

Wet Weight and DNA Content of Tissue Recombinants

Since SVM + SVE and UGM + SVE recombinants have been described previously (Cunha, 1972; Higgins et al, 1989a), emphasis will be placed on the BUG-M + SVE recombinant. The representative wholemount pictures of semitransparent tissue recombinants of BUG-M (from a single BUG) plus SVE grown for 1, 3, or 4 weeks, and BLM plus SVE grown for 4 weeks under the renal capsules of adult male mice are shown in Figure 2. In BUG-M + SVE tissue recombinants grown *in vivo* for 1 week (Fig. 2A), epithelium was completely surrounded by mesenchymal tissue and had undergone modest branching morphogenesis. When grown for 3 and 4 weeks (Fig. 2B, C), epithelium of BUG-M + SVE recombinants had under-

gone extensive branching morphogenesis. By contrast, in the BLM + SVE recombinants the SVE disappeared after 4 weeks of growth or was seen as a degenerative calcium deposit in some recombinants (Fig. 2D). The BLM remained viable and the same size throughout the period of *in vivo* growth. UGM + SVE and SVM + SVE tissue recombinants grew considerably and contained an opaque white secretion within glandular lumina after 4 weeks of *in vivo* growth as described previously (not illustrated, Cunha, 1972; Higgins et al, 1989a).

Since individual tissue recombinants were too small to measure wet weight before *in vivo* grafting, they were pooled and weighed together to calculate wet weight. The mean values of wet weight of BUG-M + SVE tissue recombinants prepared with BUG-M from a single BUG before and after 4 weeks of grafting were 0.04 mg ($n = 20$) and 1.45 ± 0.13 ($n = 13$), respectively. This constituted a 36-fold increase in wet weight during 4 weeks of *in vivo* growth. The wet weight of BUG-M + SVE tissue recombinants prepared with mesenchyme from three BUGs was 4.97 ± 0.60 mg ($n = 10$) 4 weeks after grafting. Mean wet weight of BLM + SVE recombinants before grafting was 0.07 mg ($n = 18$), which remained unchanged throughout the period of *in vivo* growth. Change in wet weight of these tissue recombinants over the time-course of *in vivo* growth is shown in Figure 3A.

The DNA contents of BUG-M + SVE tissue recombinants prepared with a single BUG-M before and after 4 weeks of grafting were 0.91 ± 0.01 μg ($n = 6$) and 24.75 ± 3.9 ($n = 6$), respectively, which represents a 27-fold increase during 4 weeks of *in vivo* growth. DNA content of the BUG-M + SVE recombinants prepared with three BUGs ($n = 6$) 4 weeks after grafting was 39.36 ± 5.98 μg . DNA content of BLM + SVE recombinants before grafting was 0.80 ± 0.03 $\mu\text{g}/\text{tissue recombinant}$ ($n = 8$); this value remained unchanged throughout the period of *in vivo* growth. The time-course for the increase in DNA content in BUG-M + SVE tissue recombinants prepared with a single BUG-M and BLM + SVE is shown in Figure 3B.

Histology and Immunocytochemistry

Mouse SVE grown in association with SVM or UGM formed a complex, highly convoluted mucosa characteristic of the histodifferentiation of the SV (Fig. 4A,C). By contrast, mouse SVE grown in association with BUG-M formed branched ductal structures lined with a tall-columnar pseudostratified epithelium (Fig. 4E). Individual ductal lumina were simple and almost completely devoid of convoluted infoldings characteristic of normal SV. Considerable fibromuscular stroma intercalating among glandular structures was seen in the BUG-M + SVE recombinants. Using an antibody against mouse seminal vesicle secretion (anti-mSVS), intense immunocytochemical staining was observed in the epithelium (apical cy-

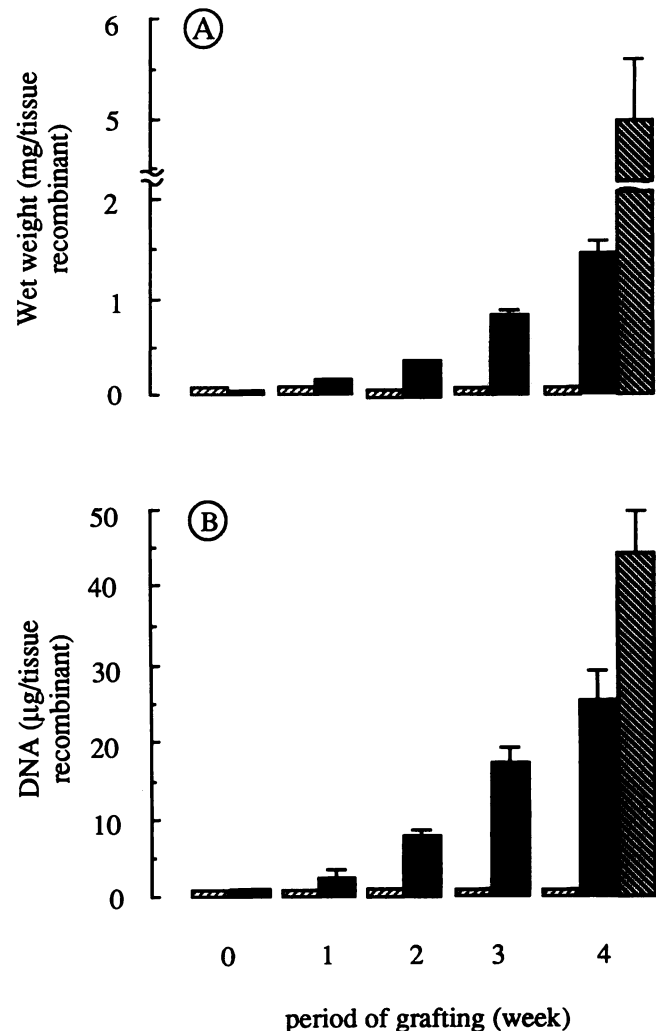


FIG. 3. Wet weight (A) and DNA content (B) of BUG-M + SVE recombinants prepared with single BUG-M (■) and BLM plus SVE (▤) before, and 1, 2, 3, or 4 weeks after transplantation to the renal site, and of BUG-M + SVE recombinants prepared with three BUGs and grown for 4 weeks (▨). Note increase in wet weight and DNA content according to the period of growth in BUG-M + SVE recombinants. Wet weight and DNA content of BLM + SVE recombinants remained unchanged throughout the same period.

toplasm) and luminal contents of SVM + SVE and UGM + SVE tissue recombinants (Fig. 4B,D). Seminal vesicle proteins were not detected with anti-mouse SVS in BUG-M + SVE tissue recombinants either in the epithelial cells or in luminal secretion (Fig. 4F). Table 1 summarizes the data.

Electrophoretic Analysis

Analysis of proteins secreted by adult mouse BUG by SDS-PAGE revealed multiple bands (lane 1, Fig. 5A), which contrasted with mouse SV secretory proteins (lane 2, Fig. 5A). The proteins secreted from four BUG-M + SVE recombinants (lanes 3–6, Fig. 5A) were completely different from that of SV but somewhat similar to that of

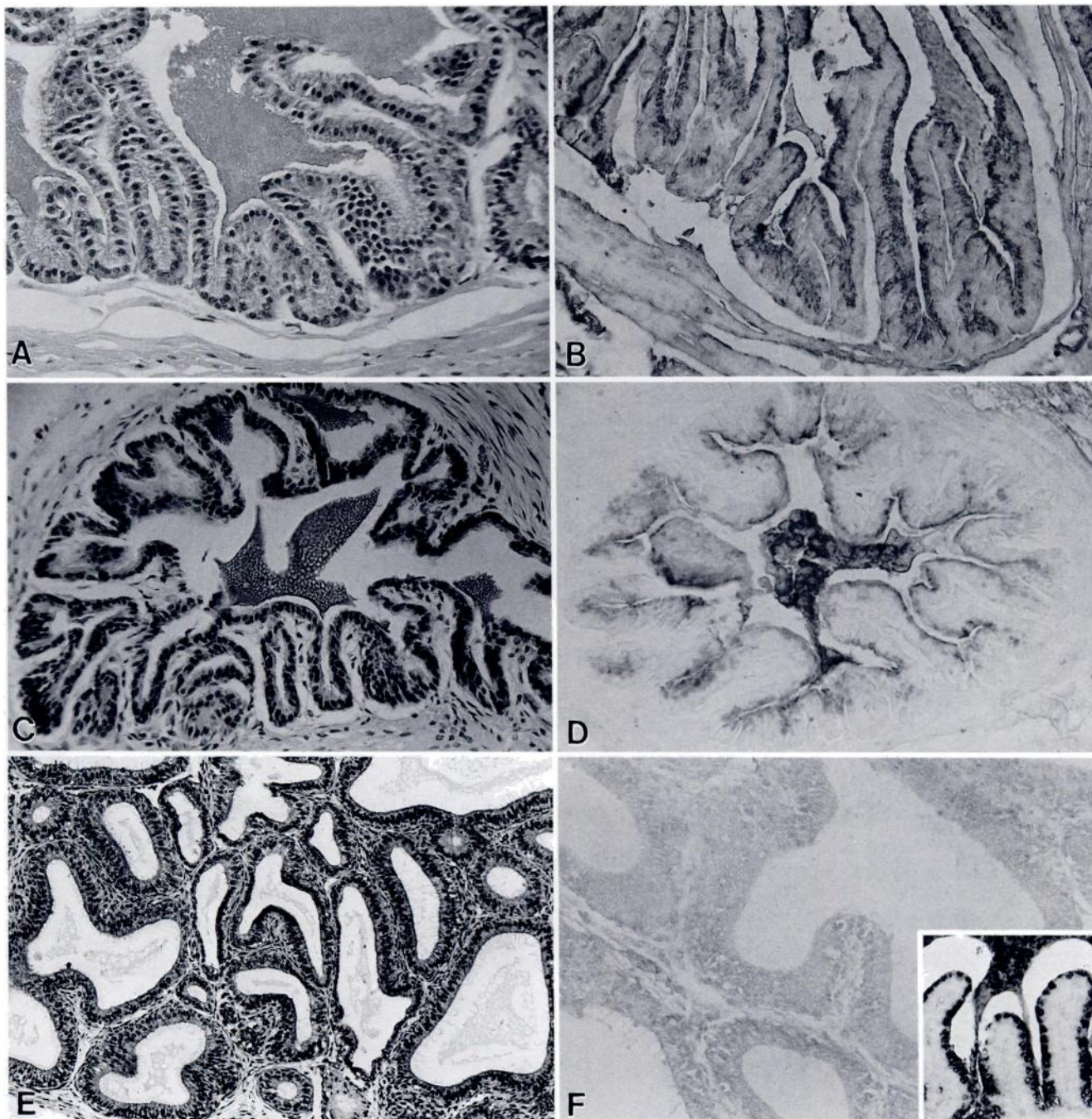


FIG. 4. Histology of (A) SVM + SVE, (C) UGM + SVE, and (E) BUG-M + SVE recombinants 4 weeks after *in vivo* grafting. In association with SVM (A) or UGM (C) a complex SV-like mucosa has formed, whereas in association with BUG-M (E) glandular structures have formed lined with tall columnar epithelial cells arranged into simple ductal structures. Immunocytochemistry using a polyclonal rabbit antibody against mouse SV secretory proteins (anti-mouse SVS) (B, D, F). Intense staining is observed in SVM + SVE (B) and UGM + SVE tissue (D) recombinants, but not in BUG-M + SVE (F) tissue recombinants. Inset in F is adult mouse SV stained with anti-SVS. (A–D and F = 200 \times , E and inset F = 100 \times .)

BUG, although several novel proteins were present. Protein patterns of BUG-M + SVE recombinants were identical whether or not 1 or 3 BUG-M were used (data not shown). Tissue recombinants composed of rat UGM + mouse SVE and rat SVM + mouse SVE expressed the major secretory proteins characteristic of the mouse SV (Fig. 5B, lanes 7–9 and 4–6, respectively).

Western Blot Analysis

By western blot analysis anti-mouse SVS, anti-MP3, and anti-mouse DLP gave specific signals for secretory proteins of the mouse SV, mouse ventral prostate, and mouse dorsal-lateral prostate, respectively, as described earlier (Mills et al, 1987; Higgins et al, 1989a; Donjacour et al,

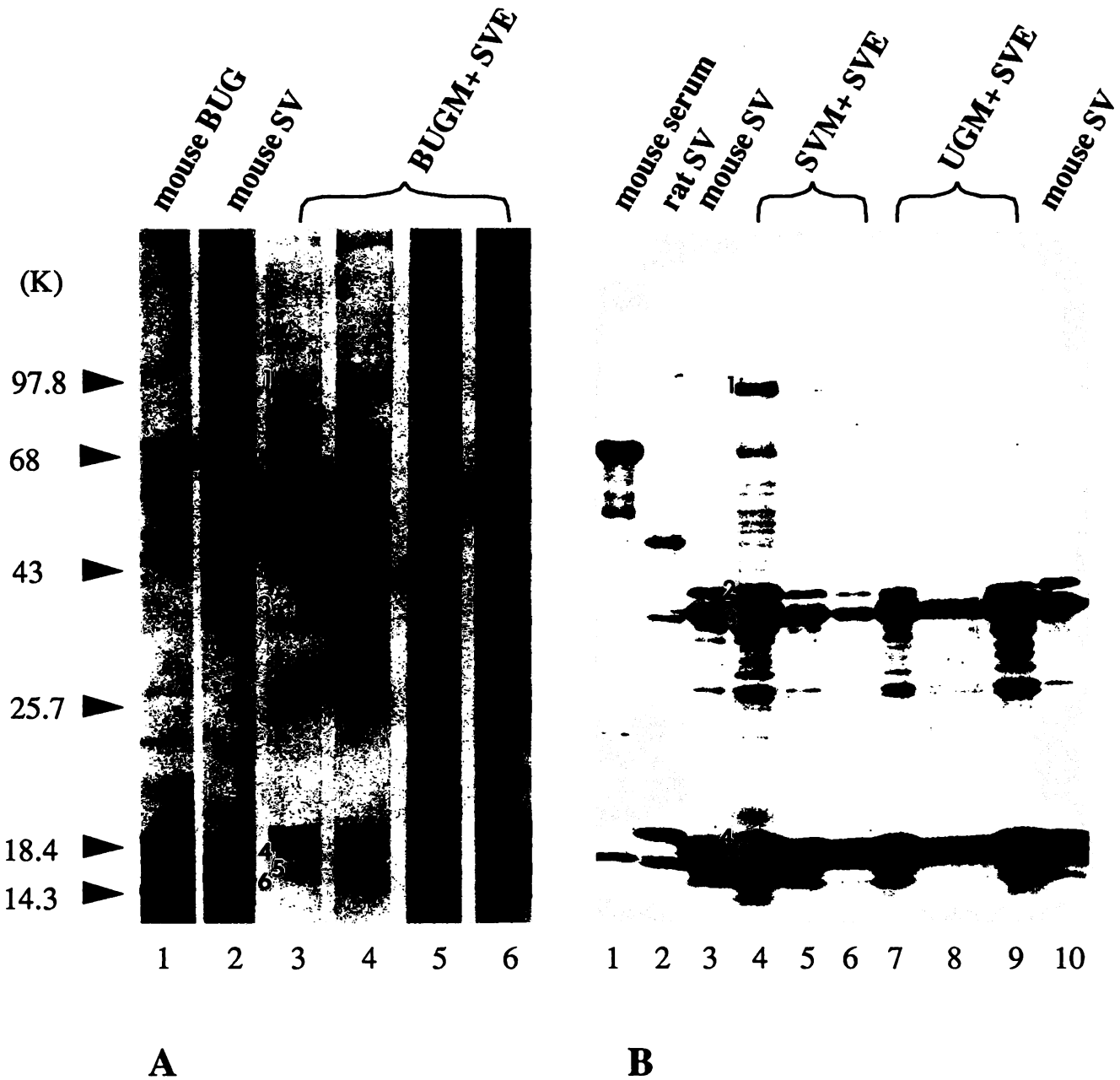


FIG. 5. Secretory proteins analyzed by SDS-PAGE. (A) Secretory proteins of normal adult BUG (lane 1), SV (lane 2), and the tissue recombinants of BUG-M plus SVE (lanes 3-6). (B) Rat SVM + mouse SVE and rat UGM + mouse SVE tissue recombinants (lanes 4-9) have expressed mouse SV secretory proteins (numbered 1-8) following 4 weeks of growth in male athymic mouse hosts. Proteins were stained with Coomassie blue. Arrowheads indicate molecular weight of standard marker proteins.

1990). None of these proteins were detected in secretions collected from BUG-M + SVE recombinants (not illustrated).

Discussion

The male urogenital tract consists in a cranial-caudal sequence of the epididymis, ductus deferens, and seminal vesicle (all of which are Wolffian duct derivatives) fol-

lowed by the bladder, prostate, and bulbourethral gland (which are derived from the urogenital sinus). The development of these organs depends on epithelial-mesenchymal interactions. In experimental situations the outcome of epithelial-mesenchymal interactions is dependent upon the germ layer origin and responsiveness of the epithelium as well as the source of the mesenchyme (see Table 2 for examples). One rule for which we have never observed an exception is that mesenchyme-induced reprogramming of epithelial differentiation is limited by the

developmental repertoire of the germ layer or anlage from which the epithelium is derived. For example, prostatic differentiation has only been elicited from endodermal epithelia, specifically endoderm of the urogenital sinus. Likewise, SV differentiation has only been induced from mesodermal epithelium derived from the Wolffian duct (Table 2). Thus, the origin of the epithelium plays a profound role in determining developmental outcome. In this report, mesenchymes normally associated with endodermal epithelia (BLM, UGM, and BUG-M) have been tested for their ability to promote development and growth of the mesodermally derived SVE.

A feature common to UGM, SVM, and BUG-M is that all are potent promoters of epithelial growth (Cunha, 1972; Chung and Cunha, 1983; Chung et al, 1984; Neubauer et al, 1986; Cunha et al, 1987; Higgins et al, 1989b; Hayashi et al, 1993). Quantitative aspects of growth in tissue recombinants have seldom been described. In this paper, BUG-M + SVE recombinants underwent considerable growth *in vivo* as judged by increase in wet weight and DNA content of the entire tissue recombinant during 4 weeks of *in vivo* growth. DNA content increased dramatically (27-fold) and appeared to be due primarily to epithelial growth. Moreover, growth was shown to be related quantitatively to the amount of BUG-M (one versus three BUG-Ms) used to construct the tissue recombinants. A similar quantitative relation between overall growth and the amount of mesenchyme utilized has been reported earlier for prostatic inductions (Chung and Cunha, 1983; Neubauer et al, 1986) and is also true for SV induction (Cunha, unpublished).

The AR status of the mesenchyme correlated with its growth-promoting activity. All three male glandular inducers (SVM, UGM, and BUG-M) express ARs (Cooke et al, 1991; Takeda and Chang, 1991) and were potent growth promoters, while BLM lacking AR did not induce the SVE to grow. Likewise, in tissue recombinants composed of adult prostatic epithelium plus UGM, epithelial growth is exuberant when wild-type UGM is used and completely absent when AR-deficient Tfm UGM is used (Norman et al, 1986; Hayashi et al, 1993). Thus, androgen-dependent epithelial growth appears to be a paracrine event mediated by AR-positive mesenchyme (Cunha et al, 1980a, 1987; Lasnitzki and Mizuno, 1980). The postulated paracrine mediators of mesenchymal-epithelial interactions in the prostate (Tenniswood, 1986) are likely to be growth factors, many of which (EGF, TGF α , TGF β , acidic and basic FGF) have been previously described in the prostate (Story, 1991). Recent studies have implicated keratinocyte growth factor as an important paracrine mediator of androgenic effects in the developing SV and prostate (Sugimura et al, unpublished data; Yan et al, 1992; Alarid et al, 1994).

Another property shared by neonatal urogenital tract

mesenchymes is the ability to reprogram competent epithelia (see Table 2). In this regard, UGM and SVM appear to have similar but not identical inductive activities. Both can induce prostatic and SV differentiation morphologically and functionally (Table 2). For example, prostatic differentiation results whether UGM (fetal prostate mesenchyme) or SVM is combined with a urogenital sinus-derived epithelium. Likewise, both SVM and UGM can induce SV histology and the expression of the full complement of SV secretory proteins in Wolffian duct-derived epithelium (Cunha, 1972; Higgins et al, 1989b; this report). Possibly, the common inductive properties of UGM and SVM can be attributed to the fact that they are located adjacent to each other and both have ARs (Cunha and Lung, 1979; Shannon and Cunha, 1983; Takeda et al, 1985; Cunha et al, 1987; Cooke et al, 1991; Takeda and Chang, 1991).

Only recently has it been appreciated that the dorsal and ventral portions of UGM have different inductive properties. This is based upon the observation that only ventral UGM is an effective inducer of ventral prostatic differentiation (Takeda et al, 1990). Presumably, dorsal UGM induces dorsal-lateral prostate although this has not been tested. SVM, while capable of inducing prostatic differentiation from epithelium of the embryonic urogenital sinus or bladder epithelium, elicits dorsal-lateral prostatic differentiation (Kimbara et al, unpublished data). Thus, one difference between UGM and SVM is that whereas UGM induces the entire prostatic complex (ventral, lateral, and dorsal lobes) during normal prostatic development (Hayashi et al, 1993), SVM is primarily a dorsal-lateral prostatic inducer, an interpretation strengthened by experiments using adult prostatic epithelium combined with SVM (Kimbara et al, unpublished data).

By contrast, BUG-M and SVM have quite different inductive properties. BUG-M did not induce the distinctive mucosal branching pattern of either the SV or BUG in SVE. In fact, the developmental response of the epithelium in BUG-M + SVE tissue recombinants is not easily characterized in the context of either permissive or instructive interactions (Saxén, 1977). The ductal structures observed in BUG-M + SVE tissue recombinants resembled prostatic tissue. Cytologically, the tall columnar epithelium of BUG-M + SVE recombinants was similar to that of the SV or the prostate and completely different from that of the adult mouse BUG, whose epithelial cells are mucinous and contain copious pale staining cytoplasm and small basal spindle-shaped nuclei. However, despite the morphological similarity to prostate, prostatic secretory proteins were not detected by western blot. Likewise, SV secretory proteins were not expressed in BUG-M + SVE recombinants as judged by immunocytochemistry, SDS-PAGE analysis, and western blots after 4 weeks

Table 2. Instructive and permissive inductions in the rodent urogenital tract

Mesenchyme*	Germ layer of epithelium		Result	Type of induction	Reference
	Epithelium	epithelium			
SVM	Prostate	Endoderm	Prostate	Permissive	(Hayashi et al, 1983; Kimbara et al, unpublished data)
VM	Urogenital sinus	Endoderm	Prostate	Permissive	(Cunha, 1975)
UGM	Urogenital sinus	Endoderm	Prostate	Permissive	(Cunha, 1972)
UGM	Prostate	Endoderm	Prostate	Permissive	(Neubauer et al, 1986; Norman et al, 1986; Hayashi et al, 1983)
UGM	Bladder	Endoderm	Prostate	Instructive	(Cunha et al, 1980b,c; Cunha et al, 1983b,c)
UGM	Urethra	Endoderm	Prostate	Instructive	(Boutin et al, 1991)
UGM	Sinus vagina	Endoderm	Prostate	Instructive	(Boutin et al, 1991)
SVM	Bladder	Endoderm	Prostate	Instructive	(Dorjicour and Cunha, 1988)
SVM	Urogenital sinus	Endoderm	Prostate	Permissive	(Cunha, 1972)
BUG-M	Prostate	Endoderm	Prostate	Permissive	(Hayashi et al, 1983; Kimbara et al, unpublished data)
SVM	Seminal vesicle	Mesoderm	Seminal vesicle	Permissive	(Higgins et al, 1989a)
SVM	Wolfian duct	Mesoderm	Seminal vesicle	Permissive	(Higgins et al, 1989b)
SVM	Epididymis	Mesoderm	Seminal vesicle	Instructive	(Turner et al, unpublished data)
SVM	Ureter	Mesoderm	Seminal vesicle	Instructive	(Cunha et al, 1991)
SVM	Ductus deferens	Mesoderm	Seminal vesicle	Instructive	(Cunha et al, 1991)
UGM	Seminal vesicle	Mesoderm	Seminal vesicle	Permissive	(Cunha et al, 1991)
UGM	Epididymis	Mesoderm	Seminal vesicle	Instructive	(Cunha et al, unpublished data)
UGM	Ureter	Mesoderm	Seminal vesicle	Instructive	(Cunha et al, unpublished data)
UGM	Ductus deferens	Mesoderm	Seminal vesicle	Instructive	(Cunha et al, unpublished data)
BUG-M	Seminal vesicle	Mesoderm	Ductal morphology	?	Current report

* SVM, seminal vesicle mesenchyme; UGM, urogenital sinus mesenchyme; VM, vaginal mesenchyme; BUG-M, bulbourethral gland mesenchyme.

of *in vivo* growth, although the expression of SV secretory proteins has been detected following as little as 2 weeks of *in vivo* growth in other heterotypic tissue recombinants (SVM + Wolffian duct epithelia) (Higgins et al, 1989a,b). Proteins collected from BUG-M + SVE recombinants were somewhat similar to those of the BUG on SDS-PAGE, but this similarity is probably due to the inclusion in the sample of common cytosolic proteins. Given the absence of antibody or molecular probes to BUG secretory proteins, the possibility that BUG-M + SVE recombinants can produce BUG secretions could not be pursued further. Nonetheless, it is unlikely that cells having such vastly different cytology could be related functionally, especially because this would imply that a mesodermally derived epithelium (SVE) would have been induced to form an endodermally derived gland (BUG), something we have never seen (see Table 2). Thus, the novel combination of BUG-M with SVE appears to have elicited the differentiation of a distinctly new phenotype that clearly is different from SV, prostate, or BUG.

In any case, BUG-M was clearly not capable of permissively inducing SV epithelial development, which was in stark contrast to UGM and SVM both of which have this capability. We predict, therefore, that BUG-M will also manifest prostatic inductive activities distinctly different from that of SVM and UGM. Indeed, while BUG-M can induce prostatic differentiation, preliminary studies suggest that BUG-M is primarily a ventral-lateral inducer (Kimbara et al, unpublished data). SVM is primarily a dorsal-lateral prostatic inducer and UGM, located spatially between the developing SV and BUG, induces dorsal, ventral, and lateral prostatic lobes. Thus, the developing male lower genital tract can be considered to contain a general glandular inductive field with variable inductive activity along its cranial-caudal (Sugimura et al, 1985) and dorsal-ventral axes (Takeda et al, 1990). Mesenchymal inductors in close proximity such as SVM or UGM on the one hand or UGM and BUG-M share common inductive activity, whereas mesenchymes at the cranial and caudal extremes of this field (SVM and BUG-M) are quite different in their inductive activities.

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