via Direct and Indirect Mechanisms

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**The inactivation of the prolactin receptor gene by homologous recombination has made it possible to investigate the role
of prolactin signaling in mammary gland development without resort to ablative surgery of the endocrine glands. In
knockout mice lacking the prolactin receptor, mammary development is normal up to puberty. Subsequently, the ducts
branch less frequently than those of wild-type animals. While terminal end buds differentiate to alveolar buds in wild-type
females by the end of puberty, in knockout females terminal end bud-like structures persist at the ductal ends. To
distinguish between the developmental defects that are intrinsic to the epithelium and those that result from systemic
endocrine alterations in prolactin receptor knockout mice, mammary epithelium from prolactin receptor knockouts was
transplanted into mammary fat pads of wild-type mice. In virgin mice, the knockout epithelial transplants developed
normally at puberty, indicating an indirect effect of prolactin on ductal development. Prolactin receptor knockout females
are infertile due to multiple reproductive defects, but epithelial transplants allowed us to assess the extent to which the
absence of prolactin receptor is limiting, under systemic conditions that allow full mammary gland development. During
pregnancy, the prolactin receptor knockout transplants showed normal side branching and the formation of alveolar buds,
but no lobuloalveolar development. Thus, prolactin affects mammary morphogenesis in two different ways: it controls
ductal side branching and terminal end bud regression in virgin animals via indirect mechanisms, but acts directly on the
mammary epithelium to produce lobuloalveolar development during pregnancy.   © 1999 Academic Press**

Key Words: **prolactin; prolactin receptor; development; mammary gland; tissue recombination.**

INTRODUCTION

The mouse mammary gland develops in discrete stages. *In utero,* a rudimentary ductal structure is produced. During puberty the resulting ducts elongate and bifurcate to fill the mammary fat pad, and ductal side branching and the formation of alveolar buds occur during each estrous cycle (Vonderhaar, 1988). During pregnancy, the alveolar buds give rise to lobuloalveolar structures capable of milk production. Following pregnancy and estrus, the gland undergoes involution with loss of most, but not all, of the epithelial components gained during the preceding event. The gland involutes further with declining ovarian function in later life.

A number of hormonal factors control these morphoge-

netic steps. Embryonic mammary epithelium develops independent of ovarian and pituitary influence (Raynaud, 1971) but is already responsive to hormonal stimuli (Ceriani, 1970). Hormonal replacement in hypophysectomized, ovariectomized, and adrenalectomized mice showed that the development of the mammary ducts is produced by a combination of growth hormone and estrogen. The addition of progesterone to this regimen causes side branching, while alveolar development resembling that of pregnancy requires the addition of prolactin (Nandi, 1958). These hormone combinations were shown to produce similar results using serum-free *in vitro* culture of whole mammary glands, although mammary development did not achieve the extent seen *in vivo* (Ichinose and Nandi, 1964; Vonderhaar, 1988), suggesting that indirect systemic effects of these hormones are important for full development.

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Targeted inactivation of genes in the mouse germ line allows dissection of the respective contributions of various hormonal factors to mammary morphogenesis. (Hennighausen and Robinson, 1998). Moreover, the application of tissue recombination techniques makes it possible to dissect the systemic effects of gene inactivation from direct effects on the target mammary tissue. In this way, it has been shown that the estrogen receptor expressed in the mammary stroma is essential for ductal elongation while its presence in the epithelium is not required at this stage (Cunha, 1997). Conversely, the progesterone receptor is required in the epithelium but not the stroma in order for ductal side branching and alveolar development to occur (Brisken, 1998).

Prolactin and other lactogenic hormones such as placental lactogen may affect mammary development directly via interaction with the prolactin receptor (PRLR), a transmembrane protein belonging to the cytokine receptor superfamily (Bazan, 1989), which is displayed by mammary epithelial cells. Prolactin may also act indirectly via its ability to regulate the function of other endocrine organs responsible for producing mammotrophic factors. Once released, these factors may act in synergy with prolactin to control mammary development. In the ovaries, for instance, prolactin and related lactogenic hormones provide trophic support to the corpora lutea, allowing estrogen and progesterone production (Galosy and Talamantes, 1995). In the liver prolactin regulates the output of insulin-like growth factor-1 (Wennbo, 1997).

Genetic ablation of the PRLR results in mice which show multiple defects in reproduction leading to infertility, altered maternal behavior, and reduced bone development (Ormandy, 1997a; Lucas, 1998; Clement-Lacroix, 1999). In the present work, we have utilized these mice to examine the role of prolactin-mediated signaling in mammary gland development. Tissue transplantation techniques were exploited to determine which of the observed abnormalities in the knockout mice can be ascribed to a direct effect of prolactin on the mammary epithelium and which are due to the loss of the PRLR from other tissues of the mouse.

MATERIALS AND METHODS

PRLR-Deficient Mouse

The PRLR-deficient mice were generated by replacement of exon 5 (Ormandy, 1998), which encodes cysteine residues essential for ligand binding and receptor activation with the NEO cassette (Ormandy, 1997a). Knockout (PRLR$^{-/-}$), heterozygous (PRLR$^{+/-}$), and wild-type (PRLR$^{+/+}$) mice used in these experiments were derived from chimeric animals made using E14 embryonic stem cells (129/OlaHsd) bred to either 129/SvPas or C57Bl6 mice and were housed in 12-h day/night cycle at 22°C and 80% relative humidity with food and water *ad libitum.*

Histology

Tissues were fixed in 10% neutral buffered formalin. Whole mounts were performed as described (Medina, 1973) using hema-toxylin or carmine alum staining. Formalin-fixed specimens and whole-mount specimens soaked in toluene to remove methylsaly-cilate were paraffin embedded and serially sectioned at 5 μm prior to hematoxylin–eosin (H&E) staining. Specimens were photographed and analyzed using a Leica MZ-12 or Leica DMRB microscope fitted with a Sony 3CCD video camera coupled to a Leica Q500MC image analysis program running on a PC.

Transplants of Mammary Epithelium

Transplants were performed as described (DeOme, 1959). Briefly, mammary gland fragments of 1 mm diameter from 8-week-old 129Ola/12SVPas knockout or wild-type mice were transplanted into the cleared fat pads of 3-week-old 129SV/C57Bl6 RAG1$^{-/-}$ mice (Mombaerts, 1992) purchased from The Jackson Laboratory (Bar Harbor, ME). Transplants were analyzed by whole-mount microscopy and histology at 10 weeks after surgery or within the first day postpartum.

Terminology

Mammary gland structures are described using the terminology developed for the human breast by Russo and Russo (1987).

RESULTS

Mammary Gland Development in Knockout Animals

At birth, wild-type and knockout females show rudiments of indistinguishable mammary ductal architecture (data not shown). These rudiments grow slowly until the onset of puberty when terminal end buds (TEBs) form and ductal elongation and bifurcation begin. Examination of the mammary glands at 14 weeks of age (Figs. 1A and 1B) shows reduced ductal side branching in knockout females. In wild-type virgins, the degree of ductal side branching increases with age (compare Figs. 1A and 1C), but in knockout animals, the ductal complexity achieved by 14 weeks remained unchanged at 32 weeks (compare Figs. 1B and 1D). Moreover, by 14 weeks of age, the TEBs of the major mammary ducts and side branches in wt animals had differentiated to yield alveolar buds (Fig. 1A). In the knockout females, TEB-like structures persist at the tips of most ducts (Fig. 1B), some of them being present at the ends of minor ducts as late as 32 weeks of age (Fig. 1D).

The persistent TEB-like structures seen at 14 weeks of age (Figs. 2B and 2D) show no resemblance to the alveolar buds seen at the ductal termini of wild-type animals at this time (Figs. 2A and 2C). Like the typical TEBs seen during ductal elongation they show direct contact between apical epithelial cells of the TEBs and stromal fat cells; however, the TEB-like structures were much smaller and contained far fewer apical cell layers with no distinct cap cell layer. These histological observations reflect their dormant behavior and indicate that the persistent TEB-like structures of knockout females were atypical.
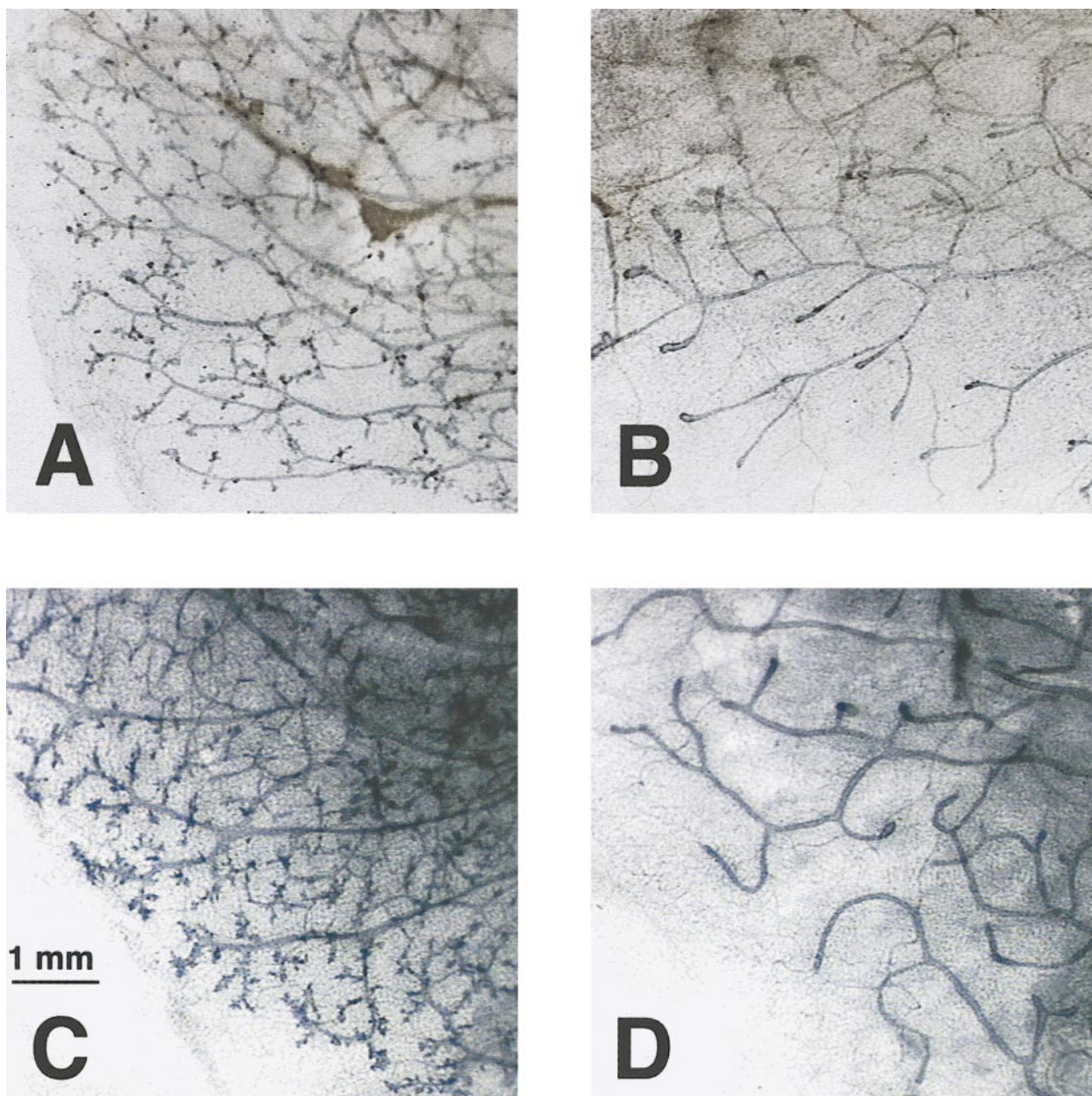
FIG. 1. Whole-mount analysis of mammary development in wild-type and knockout mice. Whole mounts of mammary glands from mice at 14 (A and B) and 32 weeks (C and D) of age were prepared from wild-type (A and C) or knockout (B and D) animals as described under Materials and Methods. Images show the dorsal portion of the fourth inguinal mammary gland. Bar indicates original size.

The male mammary gland was also investigated in animals from these litters (data not shown). No differences were observed between wild-type and knockout males in the proportion of animals with a rudimentary mammary ductal system (7/12 wild-type vs 8/13 knockout with epithelium) or the extent of ductal development.

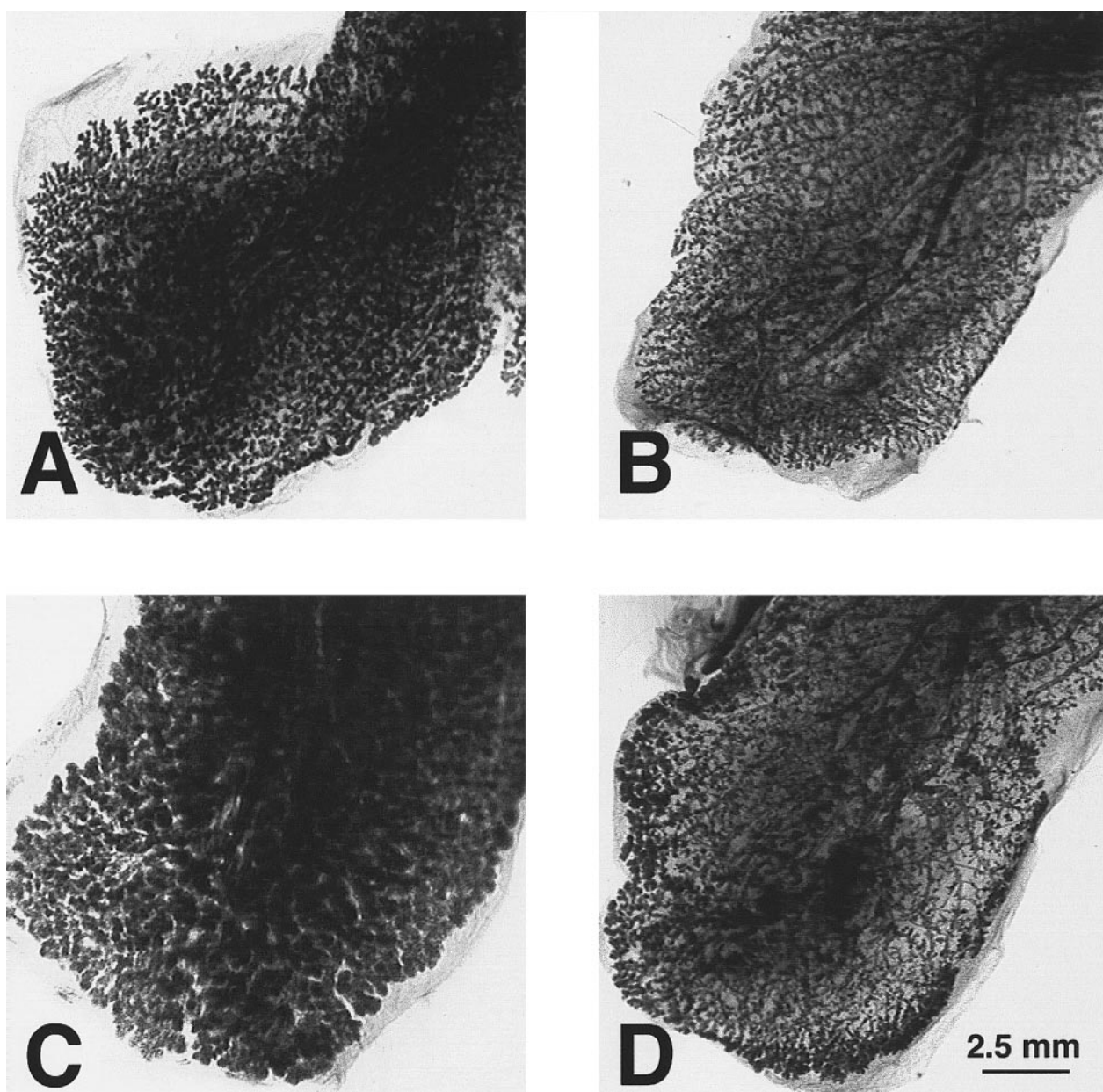### Mammary Development in Heterozygous (PRLR$^{+/-}$) Animals during Pregnancy

Heterozygous (PRLR$^{+/-}$) females, carrying just one targeted allele of the PRLR, undergo a normal pregnancy but are unable to lactate following their first pregnancy. This effect is generally lost following the second pregnancy
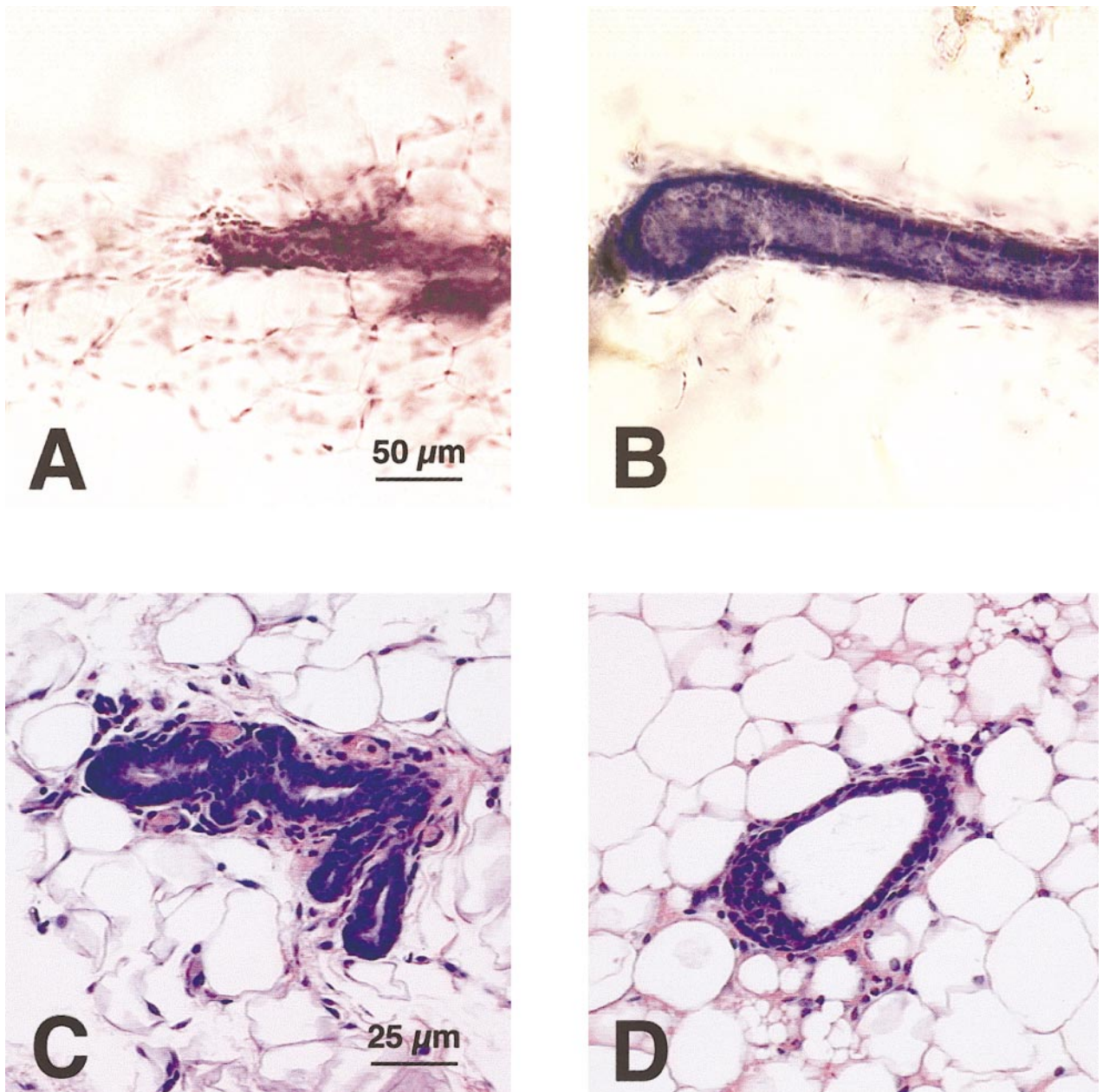
FIG. 2. Terminal ductal structures in wild-type and knockout mice. Ductal termini from whole mounts of mammary glands of virgin 14-week-old wild-type or knockout animals were microdissected and photographed at 200× original magnification under a conventional microscope in a drop of methysalycilate. (A) Ductal termini from wild-type animal showing typical alveolar buds. (B) Ductal terminus from a knockout showing persistent TEB-like structure. Terminal ductal structures from were also examined using H&E staining of 5-μm sections form wild-type (C) showing a typical alveolar bud or knockout (D) showing a TEB-like structure. Bars indicate magnification.

(Ormandy, 1997a). The basis for this observation was investigated using whole mounts and H&E-stained histological sections. Up to midpregnancy, ductal elongation, branching, and the number of lobules formed were indistinguishable between heterozygous and wild-type genotypes (data not shown). However, by day 15 of pregnancy, a substan-tially greater development of the lobuloalveoli became apparent in the wild-type females (Figs. 3A and 3B). By 1 day postpartum, the mammary glands of heterozygous females showed mostly lobules of stage 2 and 3, with a few lobules of stage 4 at the periphery of the fat pad. In contrast, the mammary glands of wild-type females contained fat pads

FIG. 3. Mammary development in PRLR heterozygous and wild-type animals during their first pregnancy. Wild-type animals (A and C) or heterozygous (PRLR$^{+/-}$) animals (B and D) were mated at 6 weeks of age and their mammary glands were analyzed by whole-mount histology at day 15 of pregnancy (A and B) or 1 day postpartum (C and D). Observation of the mother's nipples and stomach contents of the pups showed that the heterozygous animals failed to lactate despite the pups attaching to the nipple, while the wild-type animals were able to lactate fully. Bar indicates magnification.

that were densely packed with stage 4 lobules (Figs. 3C and 3D). This phenotype is not fully penetrant, with some females capable of partial lactation following their first pregnancy. The mammary glands of these heterozygous females showed many more stage 4 lobules than the glands of animals unable to lactate, but fewer than seen in wild-type females (data not shown).

Microdissection of stage 3 lobuloalveoli from the periphery of mammary glands from nonlactating heterozygous females 1 day postpartum revealed the formation of multiple alveoli, but unlike wild-type alveoli (Fig. 4A), these alveoli failed to engorge with milk (Fig. 4B). Analysis of H&E-stained serial sections (Figs. 4C and 4D) demonstrated that although the diameter of the alveoli in
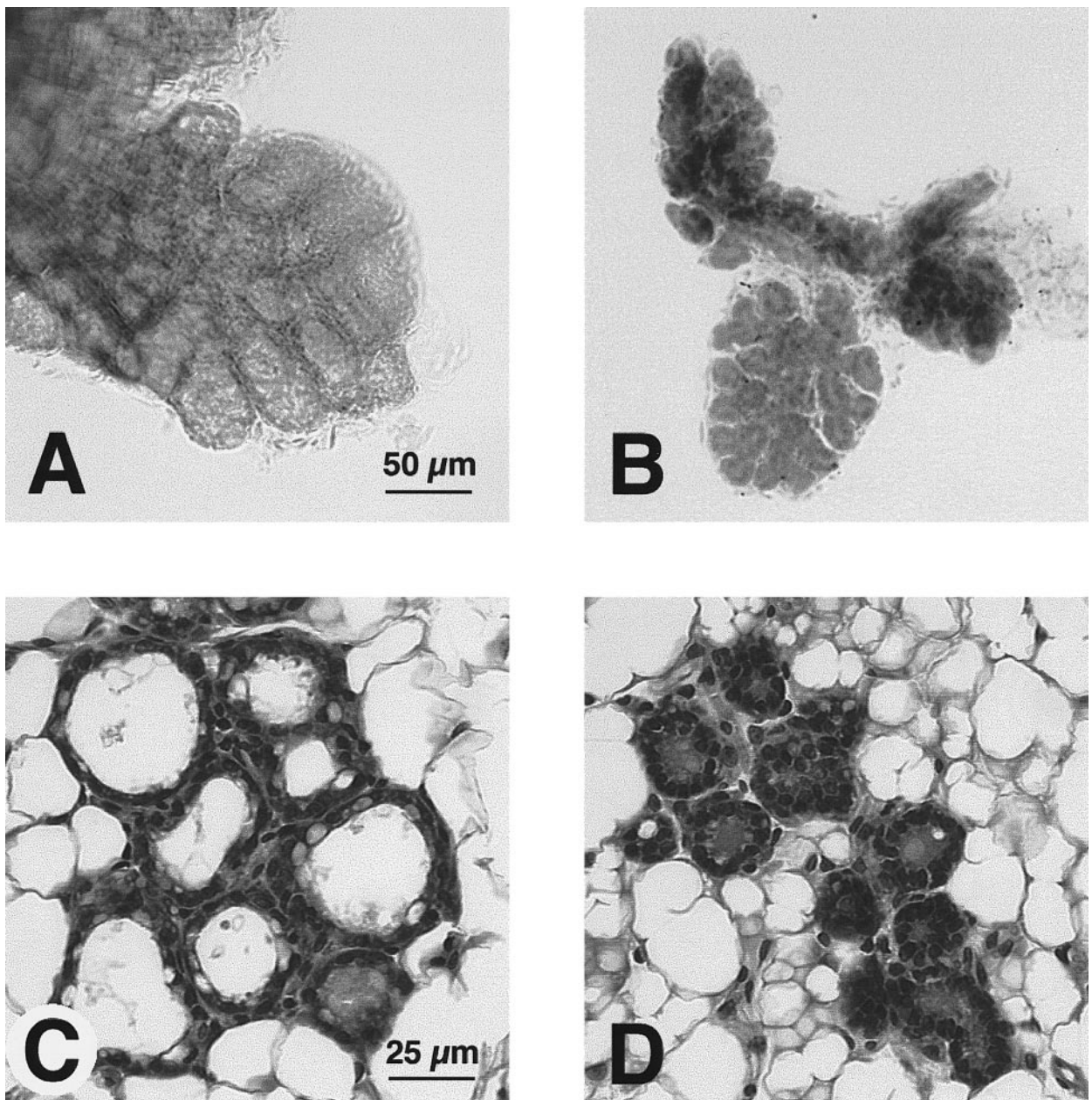
FIG. 4. Histology of mammary alveolar lobules from PRLR heterozygous and wild-type animals 1 day postpartum. Lobules from whole mounts of wild-type or heterozygous (PRLR$^{+/-}$) mammary glands were microdissected and photographed as before. (A) Wild-type lobuloalveoli type 4, 1 day postpartum, lactating normally. (B) Heterozygous lobuloalveoli type 3, unable to lactate, 1 day postpartum. The lobular histology was also analyzed using H&E staining of 5-μm sections. (C) Wild-type lobuloalveoli type 4, lactating normally. (D) Heterozygous lobuloalveoli type 3, unable to lactate. Bars indicate magnification.

heterozygous animals was smaller, they contained a similar number of epithelial cells to those of wild-type animals, indicating a failure of the final stage of functional differentiation, supported by the observation that the heterozygous alveoli contained no secretions and the epithelial cells lining these alveoli gave no evidence of the intracellular vacuoles associated with secretory activity (Figs. 4C and 4D). These results indicate that the loss of one copy of the prolactin receptor gene causes retarded mammary development, rather than a block at a
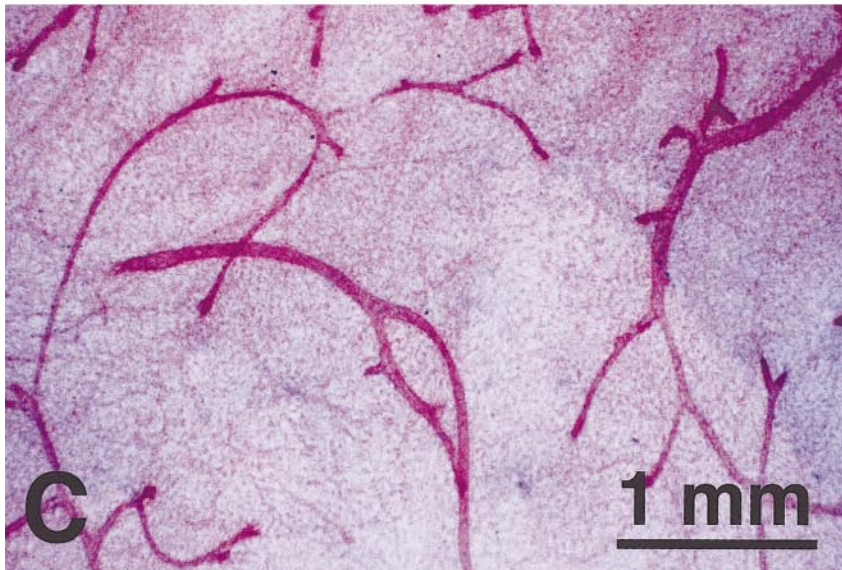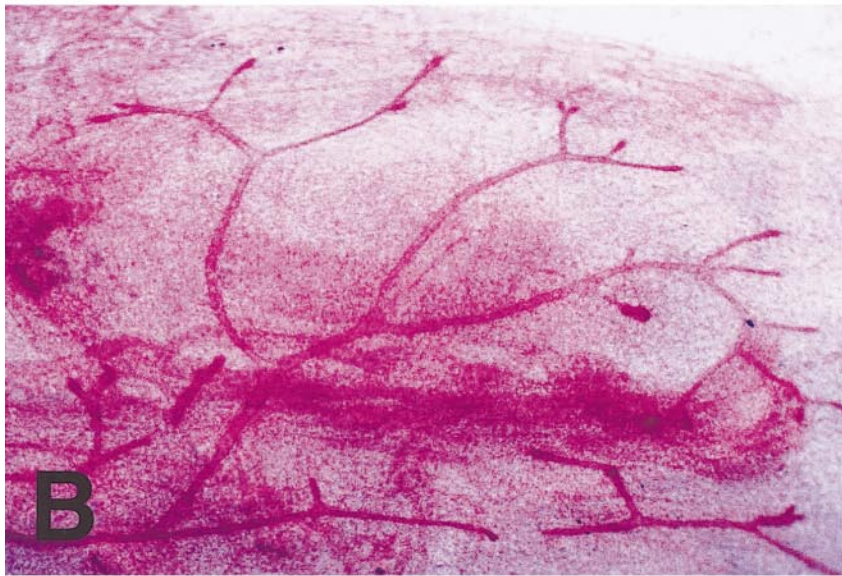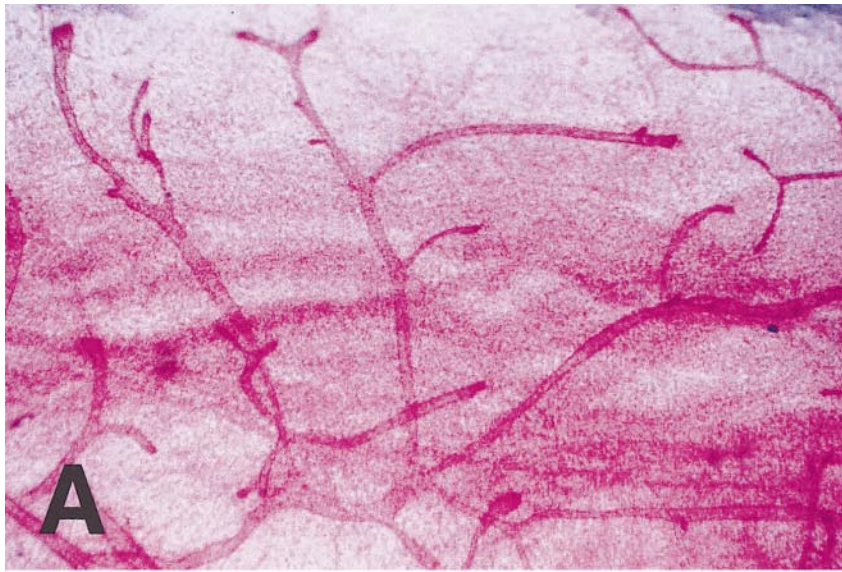
FIG. 5. Transplantation of wild-type and knockout mammary epithelium to virgin host animals. Whole-mount preparations of mammary glands from virgin RAG1$^{-/-}$ recipients 10 weeks after surgery. (A) Transplanted knockout mammary epithelium. (B) Transplanted wild-type mammary epithelium. (C) Endogenous fifth inguinal mammary gland. Bar indicates magnification.
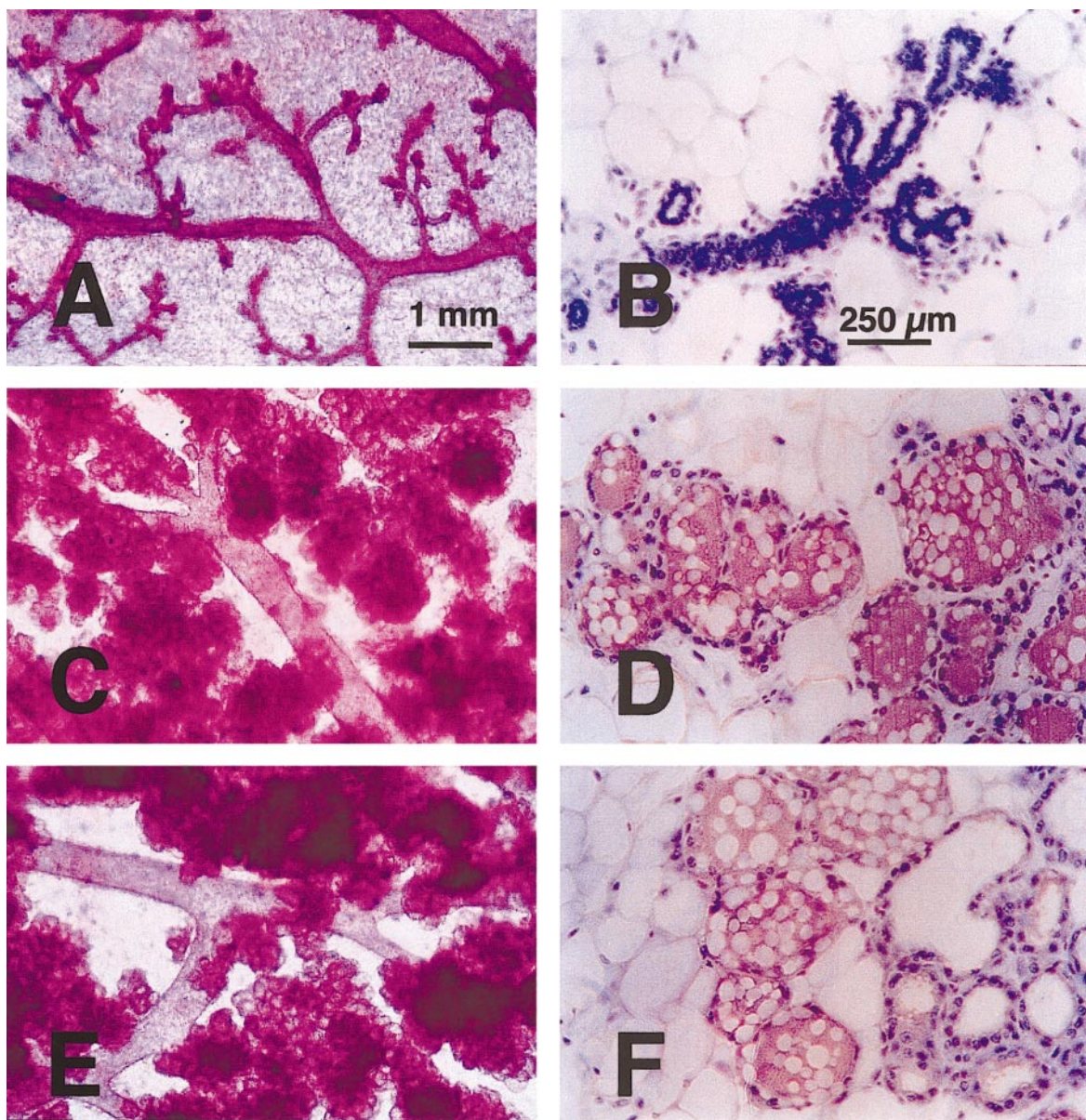
FIG. 6. Whole-mount analysis postpartum of wild-type and knockout epithelium transplanted to normal host mammary fat pad. Mammary epithelium from an 8-week-old knockout animal (A and B) or wild-type animal (C and D) was transplanted into the fourth inguinal mammary fat pad or contralateral fat pad of a 3-week-old RAG1$^{-/-}$ recipient. The recipient was mated at 10 weeks, and transplants, and an endogenous fifth inguinal mammary gland (E and F), were analyzed using whole-mount histology (A, C, and E) and sections stained for casein (Briskin, 1998) (luminal red-brown staining) (B, D, and F) on the first day postpartum. Bars indicate magnification.

particular stage of development, in contrast to the situation in knockout animals (see below). Given that most of the mammary epithelial component involutes following weaning, the effect of a first failed lactation to enable a second successful lactation is presumably exerted by a cell population which survives involution. The proposed lobular stem cell represents a good candidate population (Kordon and Smith, 1998).

Contribution of the PRLR Outside of the Mammary Epithelium to Pubertal Development of the Mammary Gland

Knockout females show multiple reproductive abnormalities (Ormandy, 1997a) indicative of a wide range of endocrine disturbances. To determine whether the abnormalities in ductal development observed in the knockout females could be ascribed to the lack of the PRLR in the

mammary epithelium or were secondary to defects in other endocrine organs, we transplanted wild-type and knockout mammary epithelia into RAG1$^{-/-}$ recipient females. Mice homozygous for the inactivated RAG1 allele lack T and B cells and are therefore able to accept allografts (Mombaerts, 1992). Both inguinal mammary glands of the recipients were cleared of endogenous epithelium (DeOme, 1959). One side was engrafted with knockout epithelium while the contralateral fat pad was implanted with wild-type epithelium from a littermate. Ten weeks after surgery, the transplanted mammary glands as well as an endogenous gland (to control for a normal endocrine milieu) were analyzed by whole-mount microscopy (Fig. 5).

Whole-mount analysis of a series of seven successfully engrafted mice showed no differences in ductal branching between the engrafted knockout and the wild-type epithelium (compare Figs. 5A and 5B) as well as the endogenous epithelium (Fig. 5C). The TEBs had regressed in all the glands and the ductal complexity was comparable. This indicates that the PRLR is not required in the epithelial cells of the mammary gland in order for ductal development and TEB regression to occur normally. Hence, the effects on these processes observed in knockout females (Figs. 1 and 2) can be ascribed to the absence of the PRLR in other cell types or organs of the mouse.

Development of Knockout Mammary Glands during Pregnancy

The function of the PRLR expressed in the mammary epithelium in mammary gland development during pregnancy could not be assessed in knockout females as these animals are infertile (Ormandy, 1997a). To circumvent this problem, we resorted once more to transplanting knockout and wild-type epithelia into RAG1$^{-/-}$ recipients. The engrafted animals were mated 4 weeks after surgery. The recipients were sacrificed after they had given birth. Both of the transplanted glands and an endogenous gland were analyzed by whole-mount and H&E histology.

In a series of 12 successfully engrafted animals, the wild-type implants (Fig. 6C) displayed a degree of ductal branching and alveolar proliferation comparable to that seen in the unmanipulated endogenous glands (Fig. 6E). The knockout transplants showed extensive side branching but no lobuloalveolar development occurred (Fig. 6A). Histological sections of transplanted and endogenous glands show that the transplanted wild-type epithelium, like the endogenous epithelium, gave rise to functional alveolar structures as indicated by the presence of secretory material in the alveolar and ductal lumina and secretory vacuoles in the epithelial cytoplasm (Fig. 6D). These morphological features of functional differentiation were completely absent from the knockout epithelium (Fig. 6B), and this was confirmed by staining for β-casein, which was not present in knockout transplants. The alveolar buds present in the knockout transplants showed a histological appearance indistinguishable from those seen in non-pregnant glands from mature animals. Taken together, these observations indicate that the PRLR expressed by mammary epithelial cells is not required for ductal growth and side branching, these effects being regulated by PRLRs in other tissues, but

is essential for lobuloalveolar proliferation and the functional differentiation of mammary epithelial cells during pregnancy.

DISCUSSION

The prolactin receptor knockout mouse has proven very useful in determining the contributions of prolactin to the development and physiology of various systems (Ormandy, 1997a; Lucas, 1998; Clement-Lacroix, 1999); however, the analysis of the mammary phenotype has been complicated by the fact that several reproductive functions affecting mammary gland development are altered in the knockout female mice. Thus, the pattern of estrous cycles is changed, no pseudopregnancy occurs, and the females are infertile. Consequently, the abnormalities observed in mammary development and physiology in knockout female mice may be due either to systemic endocrine effects or to the inability of the mammary epithelium to respond to prolactin stimulation. Here, we have used transplantation of mammary epithelium to resolve these two possibilities.

Our study of knockout mice has revealed that a functional PRLR is essential for ductal development during and after puberty. Close examination of the whole mounts shows that the major ducts appear at the same density in mammary glands of all genotypes, suggesting that dichotomous branching, which is known to be estrogen dependent, is unaffected by the absence of the PRLR and that the morphogenetic defect in mutant mice reflects a failure of ductal side branching. This conclusion is supported by the failure of the ductal trees in knockout glands to increase in complexity with age. This indicates that the epithelial cells participating in dichotomous branching respond to signals different from those that engage in side branching.

We are intrigued by the persistence of TEB-like structures in the mammary glands of knockout females. These structures maintain close contact between the apical epithelial cells and stromal fat cells that is typical of normal TEBs, but unlike normal TEBs, they do not continue to advance through the mammary fat pad, having lost their mitotic cell layers. As knockout females age, most of these TEB-like structures become simple duct ends without a distinctive morphology. These aberrant structures are probably the result of the failure of the terminal end buds to differentiate into alveolar buds under the influence of prolactin and may represent an intermediate structure in which mitogenesis and ductal elongation have been suspended but differentiation into an alveolar bud has not occurred. Similar defects in ductal branching and end-bud differentiation are also seen in mice lacking prolactin (Horseman, 1997) or Stat5a (Liu, 1998).

Alterations in ductal side branching and the failure of the TEBs to regress in the glands of knockout females are not observed when knockout epithelia are grafted into wild-type fat pads, where normally branched ducts now terminate in alveolar buds. These experiments show that PRLRs expressed outside of the mammary epithelium are responsible. Wild-type 129SV mammary epithelium adopted the less branched morphology of the endogenous Rag1$^{-/-}$ glands following transplant, confirming that systemic effects control this aspect of development, as recently demonstrated

(Yant, 1998). Use of *in situ* hybridization analysis does not reveal expression of the PRLR in the mammary stromal cells of the mouse (Bera, 1994) or the rat (Meister, 1992; Ouhtit, 1993; Shirota, 1995). This suggests that prolactin is unlikely to act via the mammary stroma in rodents. Instead, the indirect effects of prolactin on mammary gland development are likely to be traced to its role in governing the endocrine system.

A likely candidate hormone is progesterone, a hormone essential for ductal side branching (Brisken, 1998). Progesterone levels are lower in knockout females than in wild-type mice at estrus (6.8 ng/ml compared to 17.9 ng/ml) (Clement-Lacroix, 1999). We speculate that the absence of side branching in the knockout females is due to reduced ovarian progesterone production in these animals. Interestingly estrogen levels are also lower in knockout females than in wild-type females at estrus (37 pg/ml compared to 53 pg/ml) (Clement-Lacroix, 1999), but ductal bifurcation appears to be normal despite this difference. Thus again the process of ductal bifurcation may be distinct from ductal side branching.

Both prolactin and progesterone are necessary for lobuloalveolar development at pregnancy. Transplantation experiments showed that alveolar growth and differentiation during pregnancy are strictly dependent on the presence of the PRLR in the mammary epithelial cells. The complete absence of lobuloalveolar development in knockout epithelia also indicates that placental lactogens must exert their lactogenic effects directly on the mammary epithelial PRLR and not via putative lactogen receptors.

Comparison of transplanted knockout mammary epithelium to that prepared from progesterone receptor-negative mice indicates that side branching requires the presence of the progesterone receptor but not the prolactin receptor, while both receptors are essential for alveolar development. Our existing work indicates that the progesterone receptor needs to be present in ductal cells near to the alveoli but is not required in the epithelial cells of the alveoli per se (Brisken, 1998). This suggests that progesterone acts to induce paracrine signals that help to initiate or organize alveologenesis. Progesterone and prolactin interact in a number of ways to control alveolar development. First, progesterone increases expression of the prolactin receptor while prolactin increases the expression of the progesterone receptor in mouse mammary gland (Sakai, 1979; Edery, 1985) and human breast cancer cells (Ormandy and Sutherland, 1993; Ormandy, 1997b). This mechanism also operates in the uterus (Chilton, 1988), indicating that this may be a general mechanism allowing a synergistic interaction between these hormones. Recently progesterone has been reported to increase Stat5a gene expression and to induce Stat5a translocation to the nucleus via association with the progesterone receptor (Richer, 1998), providing another mechanistic link between the actions of the two hormones, as Stat5a is a major mediator of PRLR signal transduction, in addition to activation by epidermal growth factor and growth hormone. This interaction may play a part in the progesterone control of ductal side branching, as the ductal side branching defect in Stat5a knockout mice persists when Stat5a knockout mammary epithelium is transplanted to normal hosts (Liu, 1998).

Much of the interaction between prolactin and progesterone remains to be elucidated. We do not know whether the two hormones act on the same target cells, whether they act in synchrony or sequentially, or whether prolactin acts directly on the cells that have been primed by the paracrine signal that was previously released in response to progesterone. Future studies using combined mutations of the progesterone and prolactin receptors may help resolve these issues.

ACKNOWLEDGMENTS

REFERENCES

Bazan, J. P. (1989). A novel family of growth factor receptors: A common binding domain in the growth hormone, prolactin, erythropoietin and IL-6 receptors and the p75 IL-2 receptor B chain. *Biochem. Biophys. Res. Commun.* **164,** 788–795.

Bera, T. K., Hwang, S. I., Swanson, S. M., Guzman, R. C., Edery, M., and Nandi, S. (1994). In situ localization of prolactin receptor message in the mammary glands of pituitary-isografted mice. *Mol. Cell. Biochem.* **132,** 145–149.

Brisken, C., Park, S., Vass, T., Lydon, J. P., O'Malley, B., and Weinberg, R. A. (1998). A paracrine role for the epithelial progesterone receptor in mammary gland development. *Proc. Natl. Acad. Sci. USA* **95,** 5076–5081.

Ceriani, R. L. (1970). Fetal mammary gland differentiation *in vitro* in response to hormones. II. Biochemical findings. *Dev. Biol.* **21,** 530–546.

Chilton, B. S., Mani, S. K., and Bullock, W. (1988). Servomechanism of prolactin and progesterone in regulating uterine gene expression. *Mol. Endocrinol.* **2,** 1169–1175.

Clement-Lacroix, P., Ormandy, C. J., Lepescheux, L., Ammann, P., Damotte, D., Goffin, V., Bouchard, B., Gaillard-Kelly, M., Binart, N., Baron, R., and Kelly, P. A. (1999). Reduced bone formation in prolactin receptor deficient mice. *Endocrinology* **140,** 96–105.

Cunha, G., Young, P., Hom, Y., Cooke, P., Taylor, J., and Lubahn, D. (1997). Elucidation of a role for stromal steroid hormone receptors in mammary gland growth and development using tissue recombinations. *J. Mammary Gland Biol. Neoplasia* **2,** 393–402.

DeOme, K., Faulkin, L., Bern, H., and Blair, P. (1959). Development of mammary tumours from hyperplastic alveolar nodules transplanted into gland-free mammary fat pads of female CH3 mice. *Cancer Res.* **19,** 515–520.

Edery, M., Imagawa, W., Larson, L., and Nandi, S. (1985). Regulation of estrogen and progesterone receptor levels in mouse

mammary epithelial cells grown in serum-free collagen gel culture. *Endocrinology* **116,** 105–112.

Galosy, S., and Talamantes, F. (1995). Luteotropic actions of placental lactogens at midpregnancy in the mouse. *Endocrinology* **136,** 3993–4003.

Hennighausen, L., and Robinson, G. (1998). Think globally, act locally: The making of a mouse mammary gland. *Genes Dev.* **12,** 449–455.

Horseman, N., Zhao, W., Montecino-Rodriquez, E., Tanaka, M., Nakashima, K., Engle, S., Smith, F., Markoff, E., and Dorshkind, K. (1997). Defective mammopoiesis, but normal hematopoiesis, in mice with targeted disruption of the prolactin gene. *EMBO J.* **16,** 101–110.

Ichinose, R., and Nandi, S. (1964). Lobuloalveolar differentiation in mouse mammary tissue *in vitro. Science* **145,** 496–497.

Kordon, E., and Smith, G. (1998). An entire functional mammary gland may comprise the progeny from a single cell. *Development* **125,** 1921–1930.

Liu, X., Gallego, M., Smith, G., Robinson, G., and Hennighausen, L. (1998). Functional rescue of Stat5A-null mammary tissue through the activation of compensating signals including Stat5b. *Cell Growth Differ.* **9,** 795–803.

Lucas, B. K., Ormandy, C. J., Binart, N., Bridges, R. S., and Kelly, P. A. (1998). Null mutation of the prolactin receptor gene produces a defect in maternal behaviour. *Endocrinology* **139,** 4102–4126.

Medina, D. (1973). Preneoplastic lesions in mouse mammary tumorigenesis. *In* "Methods in Cancer Research," pp. 3–53. Academic Press, New York.

Meister, B., Jacobsson, G., and Elde, R. (1992). Observations on the localization of prolactin receptor mRNA in rat tissues as revealed by in situ hybridization. *Acta Physiol. Scand.* **146,** 533–534.

Mombaerts, P., Iacomini, J., Johnson, R., Herrup, K., Tonegawa, S., and Papaioannou, V. (1992). Rag-1-deficient mice have no mature B and T lymphocytes. *Cell* **68,** 869–877.

Nandi, S. (1958). Endocrine control of mammary gland development and function in the C3H/He Crg1 mouse. *J. Natl. Cancer Inst.* **21,** 1039–1063.

Ormandy, C. J., Binart, N., Helloco, C., and Kelly, P. A. (1998). Mouse prolactin receptor gene: Genomic organisation reveals alternative promoter usage and generation of isoforms via alternative 3′-exon splicing. *DNA Cell Biol.* **17,** 761–770.

Ormandy, C. J., Camus, A., Barra, J., Damotte, D., Lucas, B., Buteau, H., Brousse, N., Babinet, C., Binart, N., and Kelly, P. A. (1997a). Null mutation of the prolactin receptor gene produces multiple reproductive defects in the mouse. *Genes Dev.* **11,** 167–178.

Ormandy, C. J., Hall, R. E., Manning, D. L., Robertson, J. F. R., Blamey, R. G., Kelly, P. A., Nicholson, R. I., and Sutherland, R. L. (1997b). Coexpression and cross-regulation of the prolactin receptor and sex steroid hormone receptors in breast cancer. *J. Clin. Endocrinol. Metab.* **82,** 3692–3699.

Ormandy, C. J., and Sutherland, R. L. (1993). Mechanisms of prolactin receptor regulation in mammary gland. *Mol. Cell. Endocrinol.* **91,** C1–C6.

Ouhtit, A., Morel, G., and Kelly, P. A. (1993). Visualization of gene expression of short and long forms of prolactin receptor in the rat. *Endocrinology* **133,** 135–144.

Raynaud, A. (1971). Foetal development of the mammary gland and hormonal effects on its morphogenesis. *In* "Lactation," pp. 3–29. Butterworths, London.

Richer, J., Lange, C., Manning, N., Owen, G., Powell, R., and Horwitz, K. (1998). Convergence of progesterone with growth factor and cytokine signaling in breast cancer. *J. Biol. Chem.* **273,** 31317–31326.

Russo, J., and Russo, I. H. (1987). Development of the human mammary gland. *In* "The Mammary Gland. Development, Regulation, and Function," pp. 67–94. Plenum, New York.

Sakai, S., Bowman, P. D., Yang, J., McCormick, K., and Nandi, S. (1979). Glucocorticoid regulation of prolactin receptors on mammary cells in culture. *Endocrinology* **104,** 1447–1449.

Shirota, M., Kurohmaru, M., Hayashi, Y., Shirota, K., and Kelly, P. A. (1995). Detection of in situ localization of long form prolactin receptor messenger RNA in lactating rats by biotin-labeled riboprobe. *Endocr. J.* **42,** 69–76.

Vonderhaar, B. (1988). Regulation of development of the normal mammary gland by hormones and growth factors. *In* "Breast Cancer: Cellular and Molecular Biology," pp. 252–266. Kluwer Academic, Boston.

Wennbo, H., Kindblom, J., Isaksson, O. G., and Tornell, J. (1997). Transgenic mice overexpressing the prolactin gene develop dramatic enlargement of the prostate gland. *Endocrinology* **138,** 4410–4415.

Yant, J., Gusterson, B., and Kamalati, T. (1998). Induction of strain-specific mouse mammary ductal architecture. *Breast* **7,** 269–272.