

# Ovarian steroids regulate 24p3 expression in mouse uterus during the natural estrous cycle and the preimplantation period

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## Abstract

We examined 24p3 expression in the mouse uterus at various stages of the natural estrous cycle and during the preimplantation period. The level of 24p3 mRNA appeared intensively in proestrus and estrus, then declined sharply from metestrus to diestrus. Consistent with this observation, 24p3 protein was abundant in proestrus, decreased from estrus to metestrus and declined to a very low level in diestrus. The uterine 24p3 expression closely overlapped with the estradiol ( $E_2$ ) surge in proestrus and estrus but it was suppressed when progesterone ( $P_4$ ) rose to a high level during the reproductive cycle. Neither the protein nor its message was detected in the uteri of immature mice or ovariectomized adult animals. While an injection of  $P_4$  to these animals was unable to initiate uterine 24p3 expression, administration of estrogenic steroids to these animals markedly stimulated the gene expression. Treatment of these animals with  $E_2$  together

with  $P_4$ , on the other hand, did not stimulate the gene expression. In pregnant animals (day 1 (D1)=day of vaginal plug), 24p3 mRNA remained at a high level on D1 and D2 but dropped to an almost undetectable level on D3 and D4. This was accompanied by a decrease in 24p3 protein from D1 to D2 and a decline in the protein to undetectable levels from D3 to D4. The staining patterns of both the immunohistochemical localization of 24p3 protein and *in situ* hybridization for the detection of 24p3 mRNA in the uterine sections showed that 24p3 expression took place mainly in the luminal and glandular epithelial cells of the endometrium. This together with our previous observation that 24p3 protein is found in uterine luminal fluid indicates that the protein is secreted primarily from these cells to their respective luminal surfaces during proestrus and estrus.

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## Introduction

24p3 cDNA was originally cloned from mouse kidney culture cells infected with polyoma virus or simian virus-40 (Hrabra-Renevey *et al.* 1989). Because, normally, there is a very low level of 24p3 mRNA in kidney cells, it is suspected that overexpression of this gene might be important in the virus-induced mitogenesis. Based on a computer-assisted homologous search, the protein derived from 24p3 cDNA (hereafter referred to as 24p3 protein) is classified as a member of the lipocalin family (Flower *et al.* 1991). The protein in the lipopolysaccharide-stimulated PU5.1.8 macrophage is suggested to function in the defense against infectious agents (Meheus *et al.* 1993). The protein is also present in basic fibroblast growth factor-stimulated 3T3 cells (Davis *et al.* 1991). The study of Liu and Nilsen-Hamilton (1995) reveals that 24p3 protein is an acute phase protein of the liver.

Rodents are used as important experimental animals to study the reproductive biology of mammals. It is well

known that uterine luminal fluid (ULF) is accumulated during the proestrous phase of the rodent reproductive cycle (Albers & Neves e Castro 1961). Recently, we have demonstrated the presence of 24p3 protein in mouse ULF (Chu *et al.* 1996). It is a 25 kDa glycoprotein with a blocked N-terminus of pyroglutamate (Chu *et al.* 1997). Since 24p3 mRNA is present in both non-sexual organs and the reproductive tracts of adult mice (Chu *et al.* 1996), pathways other than the one associated with the viral infection are suspected to be involved in the regulation of 24p3 expression. This work was conducted to gain a better understanding of 24p3 expression in the mouse uterus. Our results indicate that 24p3 expression takes place mainly in the endometrial epithelial cells and that it is stimulated when serum estradiol ( $E_2$ ) concentrations are elevated during the natural estrous cycle and the preimplantation period. Our results also show that the estrogen-stimulated uterine 24p3 expression is inhibited when serum progesterone concentrations are elevated to a high level.

## Materials and Methods

### Materials

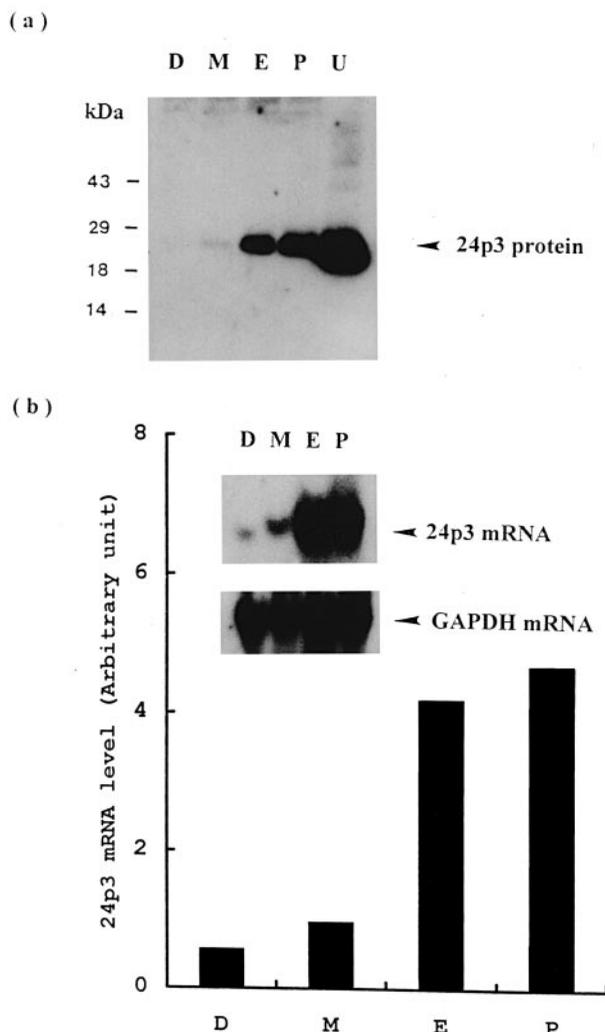
17 $\beta$ -Estradiol (E<sub>2</sub>), progesterone (P<sub>4</sub>), diethylstilbestrol dipropionate (DES), alkaline phosphatase (AP)-conjugated goat anti-rabbit immunoglobulin-G (IgG), nitro blue tetrazolium (NBT), 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma Chemical Co. (St Louis, MO, USA). [ $\alpha$ <sup>32</sup>-P]dATP and <sup>125</sup>I-labeled anti-rabbit IgG prepared from donkey were obtained from Amersham Searle (Arlington Heights, IL, USA). A DIG RNA labeling kit (SP6/T7) and anti-Digoxigenin (Dig)-AP Fab fragments were from Boehringer Mannheim GmbH (Mannheim, Germany). All other chemicals were of reagent grade. 24p3 protein was purified from mouse ULF according to our previous procedure (Chu *et al.* 1996). The antiserum to 24p3 protein was raised in New Zealand white rabbits and was used in Western blot procedures and immunohistochemical studies throughout this work.

### Animals and steroid hormone treatment

Outbred ICR mice (Charles River Laboratories, Wilmington, MA, USA) were supplied by the Animal Center, College of Medicine, National Taiwan University. Animals were kept under conditions following the institutional guidelines for the care and use of experimental animals. They were housed under controlled lighting (14 h light, 10 h darkness). Immature (21 days old) and sexually mature (6 to 8 weeks old) female mice were used for the study. The estrous cycle was staged by examining vaginal smears. The presence of a vaginal plug after mating was designated as day 1 of pregnancy (D1). The uteri were excised between 0900 and 1000 h on specified days of pregnancy (D1-D4). For the investigation of the effect of ovarian steroids, immature mice and adult female mice which had been ovariectomized one week before the initiation of steroid treatment received a daily s.c. injection of DES (30 ng/g body weight), E<sub>2</sub> (30 ng/g body weight) and/or P<sub>4</sub> (150  $\mu$ g/g body weight) in corn oil for 3 consecutive days. The control animals received corn oil only. The uteri were removed from the animals 12 h after the last injection.

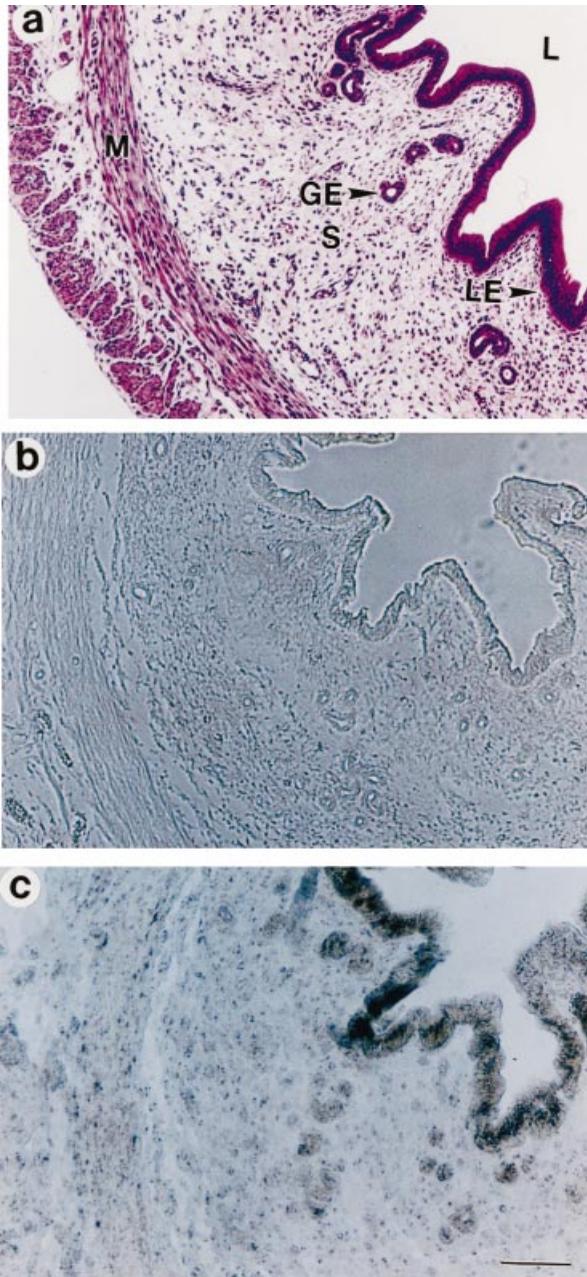
### Western blot analysis and immunohistochemical study

Mouse uteri were homogenized in phosphate-buffered saline (PBS) containing 1.0 mM PMSF and 5.0 mM EDTA, and centrifuged at 14 000 g for 10 min. The concentration of protein in the supernatant was determined by the modified Lowry method (Peterson 1977). The protein extract was resolved by SDS/PAGE on an 11% polyacrylamide slab gel (8 cm  $\times$  7 cm  $\times$  0.075 cm) according to the method of Laemmli (1970). Proteins were



**Figure 1** 24p3 mRNA and protein levels in the mouse uterus at different stages of the estrous cycle. (a) Western blot analysis of 24p3 protein. The uteri of six mature mice in diestrus (D), metestrus (M), estrus (E) or proestrus (P) were homogenated. The soluble protein extract (200  $\mu$ g) was resolved by SDS/PAGE and transferred to a PVDF membrane. The filters were immunodetected by the blot procedure with the antiserum to 24p3 protein that gave a 25 kDa band. For comparison, ULF containing 6  $\mu$ g protein was run in lane U. (b) Northern blot analysis of 24p3 mRNA. Total RNA (40  $\mu$ g) prepared from the uteri of six adult animals in the estrous cycle stages described in (a) was run on a 1.0% agarose/formaldehyde gel, transferred to a nylon membrane and probed with <sup>32</sup>P-labeled 24p3 cDNA (upper panel) and GAPDH cDNA (lower panel). The relative amounts of 24p3 mRNA that appeared as a single RNA species of approximately 1.0 kb were determined by densitometric scanning of autoradiography and adjusted with respect to the GAPDH mRNA level.

transferred from the gel slab to a polyvinylidene difluoride (PVDF) membrane (MSI, Westboro, MA, USA) in PBS at 4 °C for 20 h by a diffusion method (Bowen *et al.* 1980).



**Figure 2** Specificity of the Dig-labeled antisense 24p3 mRNA probe in the mouse uterus in the proestrous stage. (a) Tissue sections of adult female mice (8 weeks old) were stained with hematoxylin and eosin to reveal the morphology: GE, glandular epithelium; LE, luminal epithelium; M, myometrium; S, stroma; L, lumen. *In situ* hybridization was performed on the tissue sections with both the sense (b) and the antisense (c) RNA probes. Localization of 24p3 mRNA was detected by the activity staining for AP-conjugated anti-Dig Fab fragment (see Materials and Methods for details). Slides incubated with the sense probe demonstrated background staining. Photos were taken with brightfield illumination. Magnification  $\times 200$ . Bar 100  $\mu\text{m}$ .

After transfer, protein blots were immunodetected by Western blot procedures with rabbit antiserum against 24p3 protein and  $^{125}\text{I}$ -labeled anti-rabbit IgG.

Mouse uteri were fixed in Bouin's solution, embedded in paraffin and 7  $\mu\text{m}$  cross sections were mounted on gelatin-coated slides. Deparaffinized sections were blocked with 5% skimmed milk in PBS (blocking buffer) for 1 h and then treated with the 24p3 antiserum, diluted to 1:500 in blocking solution, at 4  $^{\circ}\text{C}$  overnight. After slides were washed by gentle agitation in four changes of PBS containing 0.1% Tween 20 for 15 min each, they were treated with AP-conjugated goat anti-rabbit IgG, diluted to 1:1000 in the blocking solution, for 4 h at room temperature. Slides were then washed as mentioned above and incubated for 8 min with 0.033% NBT and 0.0165% BCIP in a solution containing 100 mM Tris, 100 mM NaCl and 5 mM  $\text{MgCl}_2$  (pH 9.5). Slides were washed in four changes of water for 5 min each and the sections were then photographed with a microscope (AH3-RFCA; Olympus, Tokyo, Japan).

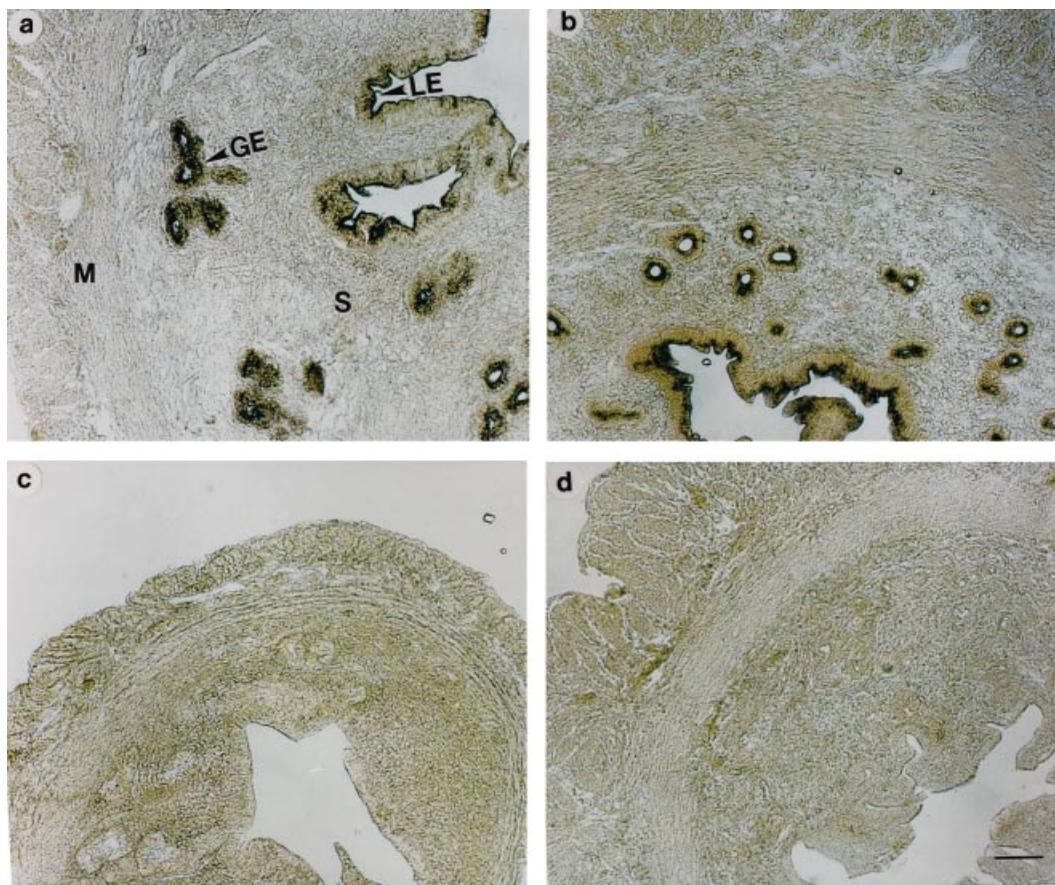
#### RNA isolation and analysis

Total RNA was prepared from mouse uteri by the standard procedure (Chomczynski & Sacchi 1987). RNA samples were analyzed by separation in denaturing 1% agarose/formaldehyde gel electrophoresis (Sambrook *et al.* 1982), followed by capillary transfer to nylon membrane. A segment from 25 to 625 bp in 24p3 cDNA inserted in pGEM-7zf (Chu *et al.* 1996) or a cDNA fragment of the mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (1233 bp) inserted in pGEM3 vector was used as a template to prepare the labeled probe. Membranes were hybridized with  $^{32}\text{P}$ -labeled cDNA probe prepared with a Promega random-priming kit (Promega, Madison, WI, USA).

#### In situ hybridization

Digoxigenin (Dig)-labeled RNA probes for *in situ* hybridization to 24p3 mRNA were prepared with a DIG RNA labeling kit (SP6/T7), using the fragment of 24p3 cDNA inserted in pGEM-7zf (Chu *et al.* 1996) as a template. Antisense probe was transcribed from BamHI-digested plasmids by T7 RNA polymerase and sense probe from XbaI-digested plasmids by SP6 RNA polymerase.

A small block of mouse uteri was fixed in PBS containing 4% paraformaldehyde at 4  $^{\circ}\text{C}$  for 4 h, embedded in paraffin, and 8  $\mu\text{m}$  sections were mounted on the gelatin-coated slides. Deparaffinized sections were washed with PBS, incubated in 0.2 M HCl for 20 min, and finally treated with proteinase K (5  $\mu\text{g}/\text{ml}$ ) in PBS for 10 min at 37  $^{\circ}\text{C}$ . The slides were briefly washed with PBS, and immersed in PBS containing 0.2% glycine for 30 min at room temperature. The sections were treated with 4% paraformaldehyde in PBS, briefly washed with PBS,



**Figure 3** Immunolocalization of 24p3 protein in mouse uterus at different stages of the estrous cycle. Uteri from adult female mice (8 weeks old) were used in the study. Immunolocalization of 24p3 protein using the rabbit antiserum was carried out for cross sections of Bouin's-fixed, paraffin-embedded uteri in (a) proestrus, (b) estrus, (c) metestrus and (d) diestrus. Localization of 24p3 protein antibody was detected by AP-conjugated anti-rabbit IgG. Magnification  $\times 250$ . Bar=100  $\mu\text{m}$ . Abbreviations as in Fig. 2.

acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8.0), dehydrated in alcohol, and air-dried. Hybridization solution, which contained Dig-labeled RNA probes (1  $\mu\text{g}/\text{ml}$ ) in a mixture of 20 mM Tris-HCl (pH 8.0), 2.5 mM EDTA, 300 mM NaCl, 1  $\times$  Denhardt's solution, yeast transfer RNA (1 mg/ml), 50% deionized formamide and 10% dextran sulfate, was added to the sections on slides. The hybridization was performed in a moisture chamber in the presence of 50% formamide/2  $\times$  SSC at 50  $^{\circ}\text{C}$  for 12 h. After the hybridization, the sections were washed with TNE solution consisting of 10 mM Tris-HCl, 500 mM NaCl, and 1 mM EDTA (pH 7.6) for 10 min followed by treatment with RNase A (20  $\mu\text{g}/\text{ml}$ ) at 37  $^{\circ}\text{C}$  for 20 min. The slides were washed three times with TNE at 37  $^{\circ}\text{C}$  for 5 min each, twice with 2  $\times$  SSC containing 0.1% SDS at 60  $^{\circ}\text{C}$  for 20 min each and finally twice with 0.2  $\times$  SSC containing 0.1% SDS at 60  $^{\circ}\text{C}$  for 20 min each. The slides were immersed in the

blocking buffer at room temperature for 1 h, and treated with AP-conjugated anti-Dig Fab fragment diluted to 1:500 in the blocking buffer at 4  $^{\circ}\text{C}$  for 4 h. Further treatment of the slides and AP activity staining were carried out as described in the immunohistochemical study.

## Results

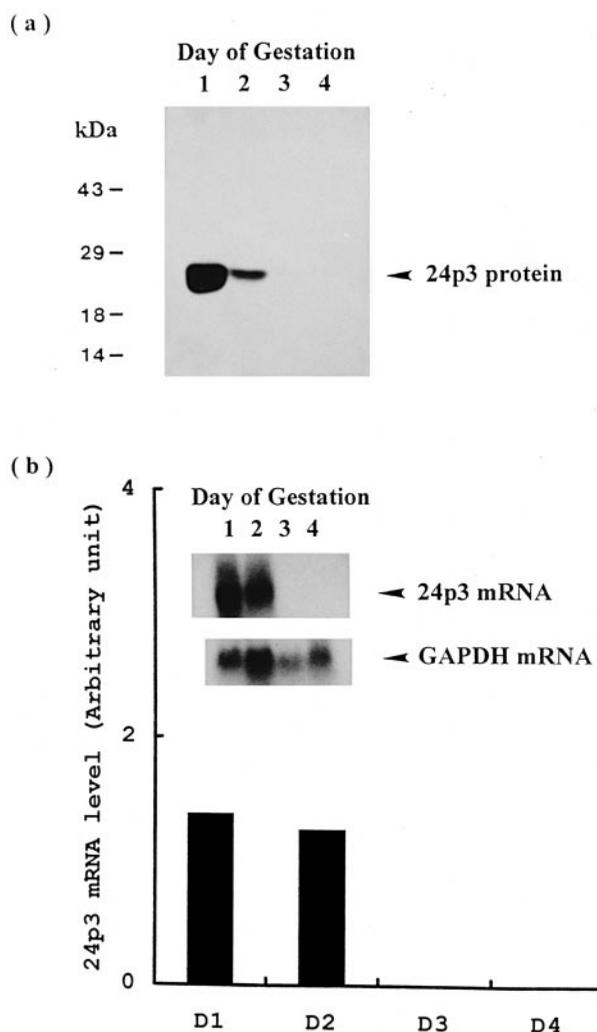
### *24p3 expression in mouse uterus during the estrous cycle*

We compared the levels of 24p3 protein and its RNA message at various stages of the estrous cycle in the mouse uteri. Figure 1b displays the result of Northern blot analysis for 24p3 mRNA in the total RNA of mouse uterus. The message appeared intensively in proestrus and estrus, then declined sharply from metestrus to diestrus. Corresponding to the change in mRNA, immunoblot detection of

24p3 protein in the soluble fractions of tissue homogenate indicated that the protein was abundant in proestrus, decreased from estrus to metestrus and declined sharply to a very low level in diestrus (Fig. 1a). The distribution of 24p3 mRNA in the uterine sections of adult mice in the proestrous stage was studied by *in situ* hybridization using the Dig-labeled 24p3 RNA probes. In contrast to the cell morphology in the tissue (Fig. 2a), the sense RNA probe gave only background hybridization and did not reveal specific localization to any cells throughout the tissue section (Fig. 2b). Although the antisense RNA probe showed rather weak hybridization to cells randomly distributed in the stroma and myometrium, the positive staining representing the location of 24p3 mRNA appeared intensively in the luminal and glandular epithelial cells (Fig. 2c), showing that 24p3 mRNA is expressed primarily in the endometrial epithelia. Immunohistochemical localization of 24p3 protein in uterine sections showed that intense protein staining was restricted to luminal and glandular epithelial cells of the endometrium but that no immunoreactivity was present in the stroma and myometrium in proestrus and estrus (Fig. 3a,b). This coincides with the distribution of 24p3 mRNA in the uterine section (cf. Figs 2c and 3a). In metestrus and diestrus, no immunoreactivity of 24p3 protein was seen throughout the uterine section (Fig. 3c,d). Based on the previous observation that 24p3 protein is found in ULF (Chu *et al.* 1996), the protein is secreted primarily from the luminal and glandular epithelial cells to their respective luminal surfaces during proestrus and estrus. Taken together, 24p3 expression in mouse uterus, which is most likely to take place in the endometrial epithelia, is enhanced in proestrus and estrus but it is not prominent in metestrus and diestrus during the natural estrous cycle.

#### 24p3 expression in the preimplantation mouse uterus

We compared the levels of 24p3 mRNA and protein in mouse uterus during the first 4 days of pregnancy. The result of Northern blot analysis shown in Fig. 4b revealed that the steady state level of 24p3 mRNA was highest on D1 and D2 and was at a very low basal level on D3 and D4. Meanwhile, immunoblot detection of 24p3 protein in the soluble fraction of tissue homogenates indicated that the immunoreactive protein band was strongest on D1, declined on D2, and disappeared on D3 and D4 (Fig. 4a). Immunohistochemical staining patterns revealed that the protein was localized in luminal and glandular epithelia and no immunoreactive cells were detected throughout the stroma and myometrium on D1 (Fig. 5a). No immunoreactivity was seen on D3 and D4 (Fig. 5c,d). On D2, the immunoreactive 24p3 protein remained intense in luminal epithelium but only very weak immunoreactivity was noted in glandular epithelium (Fig. 5b). These data indicate that in pregnant mice, the levels of 24p3 mRNA as well as 24p3 protein remain high immediately after

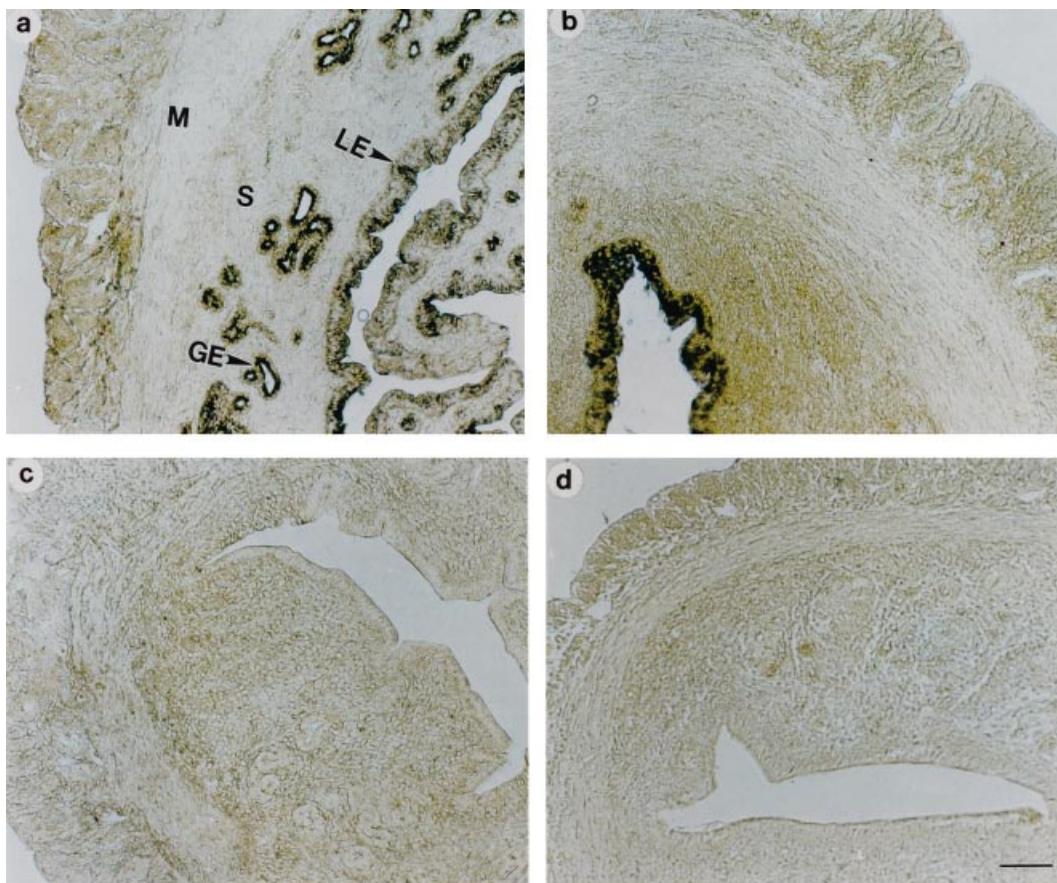


**Figure 4** Analysis of 24p3 mRNA and 24p3 protein in mouse uterus on the first 4 days (D1–D4) of pregnancy. (a) The 24p3 protein level in tissue homogenate prepared from the uteri of six animals was detected by Western blot analysis as described in Fig. 1. (b) The 24p3 mRNA level of total RNA (40 µg) prepared from the uteri of six animals was detected by Northern blot analysis. The relative amount of 24p3 mRNA was normalized with respect to the GAPDH mRNA level.

fertilization on D1 and D2, but decline sharply to almost undetectable levels with progression of pregnancy.

#### Effect of ovarian steroids on 24p3 expression in mouse uterus

The fluctuations in ovarian steroid secretion during the reproductive cycle of mature female mammals are well known. A balance between  $E_2$  and  $P_4$  is critical for many reproductive functions. This prompted us to study whether ovarian steroid hormones affected uterine 24p3 expression. In this regard, we examined how the steroid



**Figure 5** Immunolocalization of 24p3 protein in mouse uterus during the preimplantation period. Bouin's-fixed, paraffin-embedded sections were prepared from the uteri on the first 4 days of pregnancy: (a-d) D1-D4 of pregnancy respectively. Magnification  $\times 250$ . Bar=100  $\mu\text{m}$ . Abbreviations as in Fig. 2.

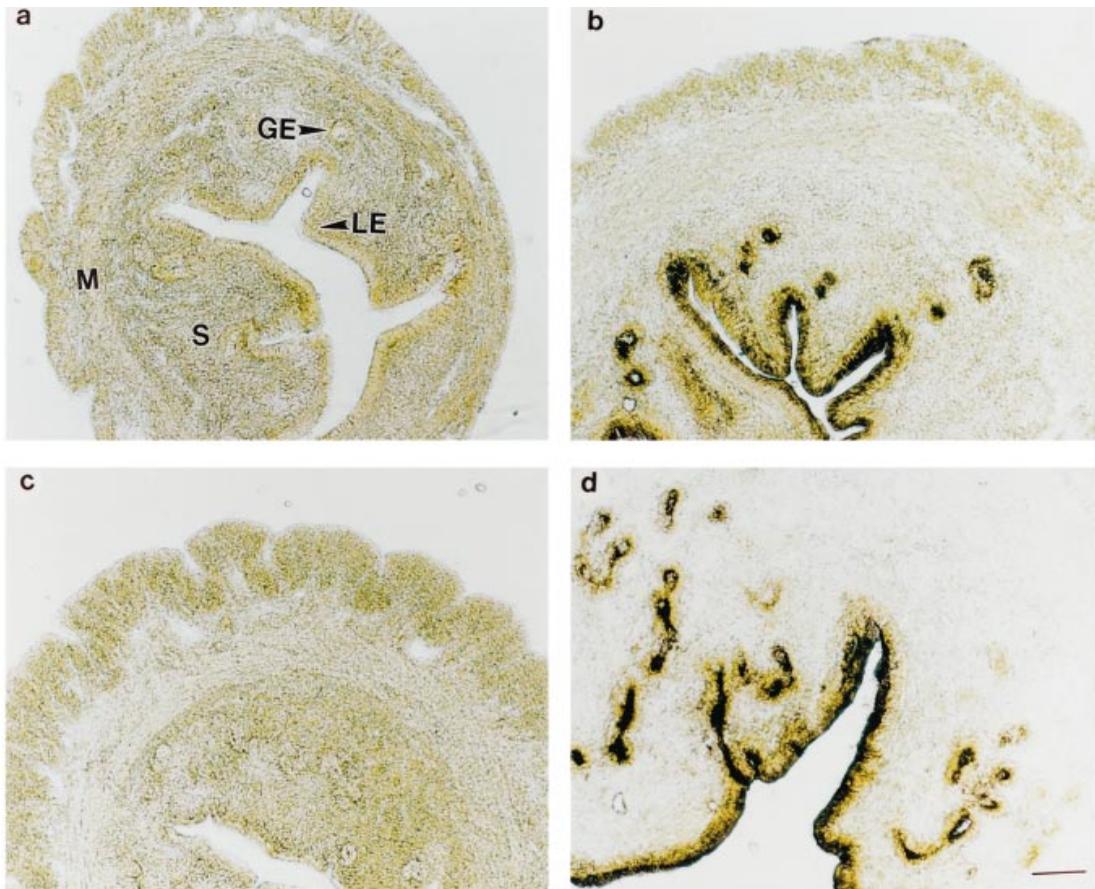
hormones affected 24p3 expression in the uteri of immature mice and ovariectomized adult mice. Figure 6 displays the immunohistochemical staining patterns of 24p3 protein in the tissue sections. While no immunoreactivity of 24p3 protein was detectable in the uteri of immature mice (Fig. 6a), intense protein staining which was restricted to luminal and glandular epithelia appeared after injection of DES (30 ng/g body weight) to the animals for three consecutive days (Fig. 6b). No 24p3 protein was present in the uteri of ovariectomized mice (Fig. 6c). Administration of either  $\text{E}_2$  (30 ng/g body weight) or DES (30  $\mu\text{g/g}$  body weight) to the ovariectomized mice stimulated 24p3 expression in the uteri as evidenced by the reappearance of 24p3 protein in luminal and glandular epithelia (Fig. 6d). These observations show that 24p3 expression is stimulated by estrogenic steroids.

24p3 mRNA was absent in the total RNA prepared from the uteri of ovariectomized mice (Fig. 7, lane 1). The message reappeared in the tissues after injection of the animals with  $\text{E}_2$  for 3 consecutive days (Fig. 7, lane 3). On

the other hand, administration of  $\text{P}_4$  (150  $\mu\text{g/g}$  body weight) alone to ovariectomized animals for 3 consecutive days was unable to restore 24p3 expression (Fig. 7, lane 2). Neither was the message detectable after administration of  $\text{P}_4$  together with  $\text{E}_2$  to ovariectomized animals (Fig. 7, lane 4), showing the antagonistic effect of  $\text{P}_4$  on the  $\text{E}_2$ -stimulated 24p3 expression in uteri.

## Discussion

Lack of 24p3 mRNA as well as 24p3 protein in the uteri of immature mice indicates the absence of uterine 24p3 expression before puberty. The loss of uterine 24p3 expression as a result of ovariectomy shows that the ovary is indispensable for the synthesis of 24p3 protein and its message in the uterus under normal physiological conditions. Our results show (Figs 6 and 7) that  $\text{E}_2$  or DES plays an important role in uterine 24p3 expression. Immunohistochemical staining patterns and the results of *in situ*

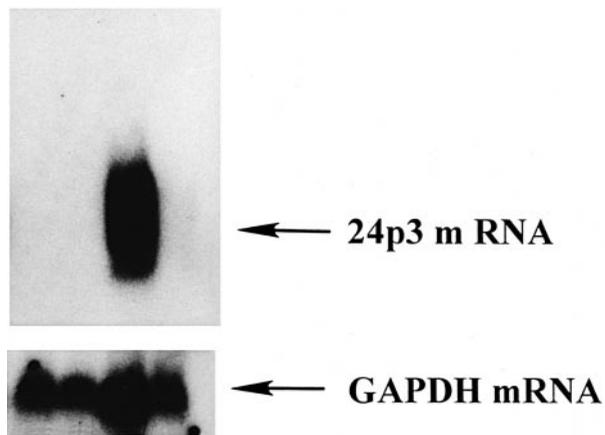


**Figure 6** Stimulation of 24p3 expression by estrogenic steroids. The cross sections of Bouin's-fixed, paraffin-embedded uteri were prepared from (a) immature mice, (b) the DES-treated ( $3 \times$ ) immature mice, (c) the ovariectomized adult mice or (d) the  $E_2$ -treated ( $3 \times$ ) ovariectomized adult mice. Magnification  $\times 250$ . Bar 100  $\mu\text{m}$ . Abbreviations as in Fig. 2.

hybridization shown in this work support the concept that 24p3 expression in the uterus most likely takes place in the glandular and luminal epithelial cells of the endometrium. Because the response of endometrial epithelial cells to both  $E_2$  and  $P_4$  is rarely maintained under *in vitro* conditions (Tomooka *et al.* 1986, Julian *et al.* 1992), our analyses of 24p3 expression in mouse uterus during the natural estrous cycle and in the preimplantation period remain essential to investigate how ovarian steroids regulate uterine 24p3 expression *in vivo*.

According to the study of Walmer *et al.* (1992), serum concentrations of  $E_2$  and  $P_4$  fluctuate between 22 and 56 pg/ml and between 2 and 16 ng/ml respectively in adult CD-1 mice. There are two surges in both  $E_2$  and  $P_4$  concentrations during the estrous cycle. A large broad  $E_2$  peak extending from late diestrus to late estrus has the highest  $E_2$  concentration in proestrus, and a small  $E_2$  peak appears at late metestrus. The  $P_4$  concentrations exhibit a small peak in early estrus and a large broad peak spanning metestrus and diestrus. Three features emerge from the

relation between the cyclic change in serum  $E_2$  and  $P_4$  and the levels of 24p3 mRNA and its protein in the uteri during the estrous cycle. First, both 24p3 mRNA and protein are expressed in association with the large broad  $E_2$  peak when  $P_4$  concentrations are low. Secondly, the level of 24p3 protein as well as its RNA message declines sharply in metestrus during which there is a small elevation of serum  $E_2$  that is accompanied with a remarkable increase in serum  $P_4$ . Thirdly, the message and the protein are barely synthesized in diestrus when serum  $E_2$  is at a basal level and serum  $P_4$  at a high level. Taken together, these results show that 24p3 expression is stimulated by  $E_2$  and that the  $E_2$ -induced gene expression can be inhibited by  $P_4$ . This mode of action can account for the revival of 24p3 expression in the uteri of ovariectomized adult mice by supplementation of  $E_2$  alone or the suppression of 24p3 expression by injection of  $E_2$  plus  $P_4$  to the animals (Fig. 7). Similar to the 24p3 expression described, estrogenic steroids regulate the expression of lactoferrin, a major ULF protein, in the mouse uterus and vagina during the estrous



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**Figure 7** Progesterone antagonism of the  $E_2$ -stimulated 24p3 expression in mouse uterus. The level of 24p3 mRNA in the total RNA prepared from uteri of ovariectomized adult mice ( $n=6$ ) which had been injected with  $P_4$  (lane 2),  $E_2$  (lane 3) or  $P_4$  together with  $E_2$  (lane 4) for 3 consecutive days was compared. Lane 1 was the control.

cycle and the preimplantation period (McMaster *et al.* 1992, Walmer *et al.* 1992).

The uterine 24p3 expression continues after fertilization until the late preimplantation period. On D1 and D2 of pregnancy when serum  $E_2$  declines to a low level and  $P_4$  concentration increases remarkably (Bridges 1984), significant amounts of 24p3 expression in the uterus were observed, presumably in response to the preovulatory estrogen surge. Thus, the gene expression in luminal and glandular epithelial cells on D1 and D2 of pregnancy (Fig. 5) may reflect the preovulatory estrogen stimulation. The intensive appearance of 24p3 protein in the luminal epithelium as compared with the weak appearance in the glandular epithelium on D2 of pregnancy may suggest that the two epithelial cell types might respond to different stimuli or have a different response to estrogen stimulus in the production of 24p3 protein. Because of either insufficient  $E_2$  or rising  $P_4$  levels in the late preimplantation period, the message of 24p3 gene declines on D3 of pregnancy and remains at a low basal level in the progression of pregnancy until it increases in quantity on the days around birth (Kasik & Rice 1995, Liu *et al.* 1997). It is of interest to note that the profile of 24p3 expression towards the end (D19 onward) of pregnancy is not only parallel with that of prostaglandin  $F_{2\alpha}$  production in the uterus (Liu *et al.* 1997) but also it is consistent with an increase in serum  $E_2$  concentration and a sharp decrease in serum  $P_4$  concentration during that period. It is well known that the preimplantation estrogen secretion on D4 is critical for

the induction of implantation in mice, but uterine 24p3 mRNA remains at the basal level on D4 (Liu *et al.* 1997). In fact, serum  $P_4$  concentration in the rat remains at a high level (80 ng/ml) on D4 according to a previous report (Bridges 1984).

Progesterone is able to antagonize the estrogen-induced uterine development and to down-regulate estrogen receptors (Hsueh *et al.* 1975). Our results document such an antagonistic effect on the  $E_2$ -stimulated 24p3 expression in the mouse uterus. Many lines of evidence indicate that the primary action of steroid hormone at the level of transcription involves the participation of a complex formed by steroid and its receptor. Such a receptor complex modulates cooperative interaction of hormone response elements and other accessory response elements in the steroid responsive genes. According to the results of Garay-Rojas *et al.* (1996), two overlapping glucocorticoid response elements (GRE) but no estrogen response element have been mapped in the 5'-flanking region of a 24p3 genomic clone which contains 793 bp in the further upstream of the 5'-flanking region, six exons, five introns and 228 bp of 3'-untranslated region. Their results demonstrate that both dexamethasone and retinoic acid can induce 24p3 mRNA expression in mouse L cells. It is possible that either there are some unidentified hormone responsive elements uncovered in the regulatory region of the 24p3 gene examined or the specificity of the two GREs is not tightly restricted for specific hormone-receptor complexes. The latter possibility cannot be neglected in view of increasing evidence that the binding of one hormone-receptor complex to its corresponding hormone-responsive elements can be competed for with another hormone-receptor complex (Glass *et al.* 1988, Beato 1989). Alternatively, the interaction between  $E_2$  or  $P_4$  with its receptor may not be involved directly in 24p3 expression but may induce some transcription factors which regulate the transcriptional activity of this gene. A further-upstream region beyond 793 bp of the 5'-flanking region may also be important. It awaits future studies to unravel the complication of hormonal effects on uterine 24p3 expression.

Alignment of the amino acid sequence of 24p3 protein and a neutrophil gelatinase-associated lipocalin (NGAL) (Kjeldsen *et al.* 1993), which is present in the specific granules of human neutrophils (Allen *et al.* 1989), reveals that the two proteins have 70% identity (Chu *et al.* 1997). We could detect 24p3 protein in the granulocytes prepared from mouse blood (Y H Chen, unpublished observation). Although the migration of polymorphonuclear leukocytes (PMN) into endometrial stroma during proestrus and the association of PMN with the epithelial cells during estrus is well known (Walmer *et al.* 1992), we found almost no 24p3 protein in the uterine stroma and myometrium during the reproductive cycle. Apparently, the 24p3 protein which is associated with the surface of luminal and glandular epithelial cells may not originate

from the secretion of PMN but may primarily come from the translation of 24p3 mRNA in the endometrial epithelia.

### Acknowledgements

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