MOLECULAR AND CELLULAR BIOLOGY, July 1987, p. 2335–2343
0270-7306/87/072335-09$02.00/0

Vol. 7, No. 7

Expression of Rat Transforming Growth Factor Alpha mRNA during Development Occurs Predominantly in the Maternal Decidua

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Received 10 February 1987/Accepted 30 March 1987

Previous studies have shown that transforming growth factor α is expressed during rodent development. To establish the site(s) of transforming growth factor α mRNA expression during rat embryogensis, we performed in situ hybridization and Northern blot analyses on samples of embryonic and maternal tissues at various gestational ages. Our results indicate that the high levels of transforming growth factor α mRNA that are observed during early development are the result of expression in the maternal decidua and not in the embryo. Decidual expression appears to be induced after implantation, peaks at day 8, and then slowly declines through day 15 at which time the decidua is being resorbed. Expression of transforming growth factor α mRNA is highest in that region of the decidua adjacent to the embryo and is low or nondetectable in the uterus, placenta, and other maternal tissues. The developmentally regulated expression of transforming growth factor α mRNA in the decidua, together with the presence of epidermal growth factor receptors in this tissue, suggests that transforming growth factor α stimulates proliferation locally through an autocrine mechanism. Since epidermal growth factor receptors are present in the embryo and placenta, transforming growth factor α produced in the decidua may also act on these tissues through paracrine or endocrine mechanisms.

Transforming growth factors (TGFs) are polypeptides, first purified from retrovirus-transformed cells in culture, that have been shown to confer on cultured normal cells phenotypic properties associated with transformation (4, 27). These reversible alterations include changes in morphology, the loss of contact inhibition, and the acquisition of growth in soft agar. Two distinct TGFs have been described: TGFα and TGFβ. TGFα is a single polypeptide of 50 amino acids that shares 30% sequence homology with epidermal growth factor (EGF) (17) and exerts its biological action by binding to the EGF receptor (5, 22). TGFβ is an unrelated homodimer of 112 amino acids that does not bind the EGF receptor (6, 18, 26). Interactions between TGFα and TGFβ are complex, with the outcome depending on the cell type examined; e.g., in fibroblasts grown in soft agar, TGFβ potentiates the mitogenic action of TGFα (and EGF) and does not act as a mitogen itself (19, 25). In view of their biological actions and the finding that human and rodent TGFs are expressed by a variety of transformed as well as tumor cells, it has been proposed that these proteins play a role in the growth of some tumors through autocrine and paracrine mechanisms (31).

cDNAs encoding both human (8) and rat (15) TGFα have recently been cloned. Sequence analyses of these cDNAs suggest that TGFα is initially expressed as a transmembrane precursor of either 160 (human) or 159 (rat) amino acids and that the proteolytic release of the mature growth factor occurs through the action of an elastaselike enzyme. The cloned cDNAs have been used to demonstrate that TGFα mRNA is expressed by many solid tumors, as well as by cultured cells transformed by retroviruses and chemicals (7; D. Lee, unpublished data). In contrast, TGFα mRNA does not appear to be expressed at significant levels by most normal tissues or nontransformed cells (14) (also see Results). An exception is the finding of expression of both TGFα mRNA and its encoded peptide during rodent development. Nexo et al. (21) showed that mouse embryos between days 11.5 and 17.5 of gestation contained more EGF-like material than could be accounted for by an EGF-specific radioimmunoassay. Using radioreceptor assays and bioassays, Twardzik (32) subsequently demonstrated a high level of TGFα in day 7 mouse embryos. This high level then declined and was followed by a smaller peak at day 13. Similarly, we have previously shown that rat embryos at days 8 and 9 of gestation contain significant levels of TGFα mRNA which then fall to nondetectable levels by day 13 (14).

Because the results of the studies cited above are not entirely consistent with each other, and because the highest level of expression appears to occur at a gestational age (day 7 in mouse) when it is very difficult to isolate the embryo free of maternal tissues, we performed Northern blot analysis and in situ hybridization on samples or at embryonic and maternal tissues. We report here that the high levels of TGFα mRNA that have been previously observed during early development are the result of expression in the maternal decidua and not in the embryo. Moreover, this expression, which is highest in that portion of the decidua immediately adjacent to the embryo, appears to be induced after implantation of the embryo, peaks at day 8 of gestation, and then slowly declines as the decidua is resorbed.

MATERIALS AND METHODS

Animals and tissues. Nonpregnant and timed-pregnant Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were sacrificed, and the uteri were removed aseptically. The age of gestation was determined from plug dates (the plug day is defined as day 0 of gestation) and from the average length of the embryo (28). The uteri, decidua, embryos, and placenta were separated surgically

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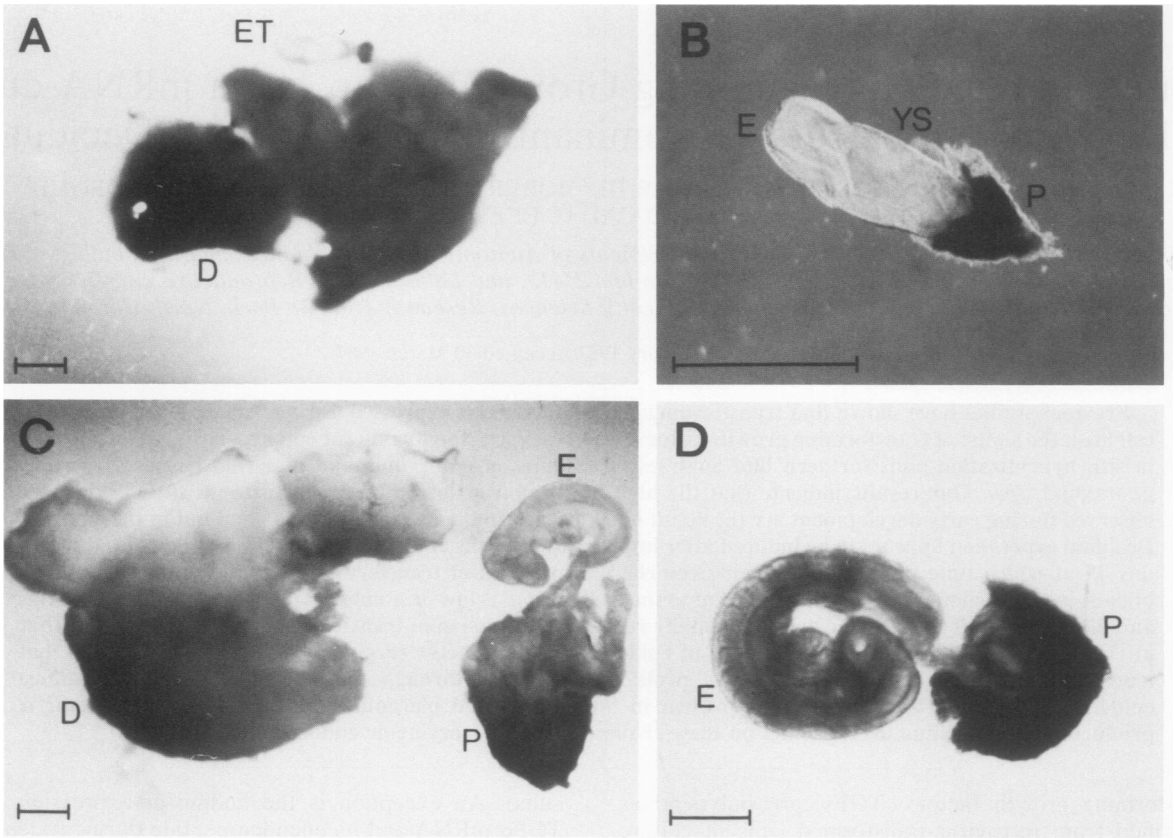

FIG. 1. Photomicrographs of embryonic and maternal tissues dissected from animals at days 8 (A and B) and 11 (C and D) of gestation. Enlargements of the embryonic tissues in panels A and C are shown in panels B and D, respectively. Note that at day 8 of gestation, the hook-shaped embryo, which is less than 1 mm in length, is isolated as an embryonic tube that contains, in addition to the embryo proper, associated membranes, yolk, sac, and developing placenta. By day 11 of gestation, the now recognizable embryo has grown to approximately 6 mm in length, contains 16 somites, and as shown, is attached to the placenta by the umbilical cord. ET, Embryonic tube; D, decidua; E, embryo; YS, yolk sac; P, ectoplacental cone (B) or placenta (C and D). Bar = 1 mm.

under a dissecting microscope (Fig. 1). As dissected, the uterus includes parametrium, myometrium, and various amounts of endometrium or decidua or both depending on the gestational age. The dissected embryo before day 10 includes not only the embryo proper but also associated membranes, the yolk sac, and the developing placenta.

For in situ hybridization, tissues were fixed immediately after disection by immersion in 2% paraformaldehyde–1% glutaraldehyde in 100 mM phosphate buffer (pH 7.0) at 4°C for 16 to 18 h. This was followed by several washes in phosphate-buffered saline over 2 days at 4°C and incubation in phosphate-buffered saline containing 30% sucrose for 2 days at 4°C (12). For poly(A)$^+$ RNA extraction, tissues were pooled from several litters, immediately frozen on dry ice, and stored at −70°C until processed.

Northern blot analyses. Total RNA was prepared from frozen tissues by homogenization in 4 M guanidine thiocyanate followed by centrifugation on 5.7 M cesium chloride (34). Poly(A)$^+$ RNA was selected by oligo(dT)-cellulose chromatography (1). Before Northern blot analyses, the quality of the poly(A)$^+$ RNAs was assessed by electrophoresis through 1% agarose–8 M urea gels followed by staining with ethidium bromide.

Poly (A)$^+$ RNA (10 μg) from each tissue was resolved by electrophoresis through 1% agarose gels containing formaldehyde (16), followed by transfer to a nitrocellulose membrane. Blots were hybridized with 10^6 cpm of nick-translated rat TGFα cDNA insert per ml (15). Hybridization was performed at 42°C in 50% formamide–5× SSC (1× SSC is 0.15 M naCl plus 0.015 M sodium citrate, pH 6.8)–1× Denhardt reagent (20× Denhardt reagent is 0.4% each bovine serum albumin, Ficoll, and polyvinylpyrrolidone)–25 mM NaH_2PO_4 (pH 7.0)–100 μg of herring sperm DNA per ml. Washes were performed in 2× SSC–0.1% sodium dodecyl sulfate at 68°C. Blots were then air dried and exposed to X-ray film (XAR-5 or XRP-2; Eastman Kodak Co., Rochester, N.Y.) with an intensifying screen at −70°C.

In situ hybridization. In situ hybridization was performed as described previously (12). Briefly, 6-μm sections of fixed tissues were obtained with a cryostat and mounted on poly-L-lysine-coated slides. Sections were washed in phosphate-buffered saline containing 0.2% Triton x-100 for 1 h, prehybridized for 2 h at 42°C, dehydrated through an ascending ethanol series, and hybridized with either 2×10^7 cpm or ^{32}P-labeled TGFα oligomers or nick-translated TGFα cDNA insert per ml or 1×10^7 cpm or ^{3}H-labeled TGFα cRNA probe per ml. Prehybridization and hybridization buffers for the ^{32}P-labeled oligomers and cDNAs contained 50% formamide, 5× SSC, 2.5× Denhardt reagent, 10% dextran sulfate, 100 μg of herring sperm DNA per ml, and 100 μg of yeast tRNA per ml. Prehybridization and hybridization buffers for ^{3}H-labeled cRNA probes contained 70% formamide, 2.5× SSC, 2.5× Denhardt reagent, 100 μg of herring sperm DNA per ml, and 100 μg of yeast tRNA per ml.

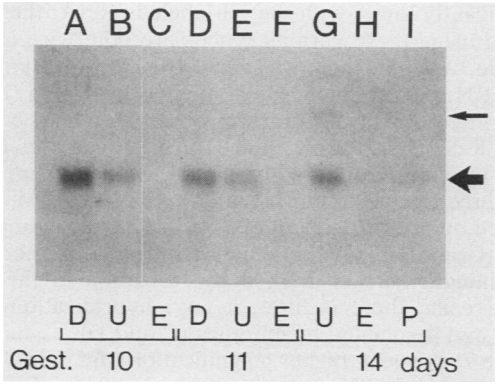

FIG. 2. Expression of TGFα mRNA is predominantly nonembryonic. Northern analysis of poly(A)$^+$ RNAs (10 μg per lane) from decidua (D, lanes A and D), uterus (U, lanes B, E, and G), embryo (E, lanes C, F, and H), and placenta (P, lane I) at days 10 (lanes A to C), 11 (lanes D to F), and 14 (lanes G to I) of gestation, probed with the cloned rat TGFα cDNA. Large and small arrows indicate the migration of the 4.5-kb TGFα mRNA and an unknown transcript of 10 kb, respectively. Gest., Gestation.

Hybridization was performed for 24 h at 42°C with the oligomers and cDNA and at 60°C with the cRNA. Sections were washed at 42°C for 2 h in 2× SSC and then at room temperature for 1 h in 0.1× SSC, dehydrated in an ascending ethanol series, and air dried. Sections were exposed to Kodak NTB-3 photoemulsion for 7 to 21 days at 4°C, developed with Kodak D19 developer, and treated with Kodak fixative. Sections were stained with hemtoxylin and eosin, dehydrated in an ascending ethanol series, mounted, and cover slipped with Permount (Fisher Scientific Co., Pittsburgh, Pa.).

The ^{3}H-labeled TGFα cRNA probes used in the in situ hybridization analyses were prepared from an SP64 vector (Promega Biotec, Madison, Wis.) containing an *Eco*RI-*Bgl*II fragment from the 5′ end of the cloned TGFα cDNA (15). This 400-base-pair fragment contains both 5′ untranslated and coding sequence. To synthesize the ^{3}H-labeled cRNA, the SP64-TGFα vector was cleaved with *Eco*RI and then transcribed in the presence of [^{3}H]UTP and [^{3}H]CTP (Amersham Corp., Arlington Heights, Ill.) according to the directions of the manufacturer. The resulting transcript was base hydrolyzed to an average length of 150 nucleotides as described by Cox et al. (3).

RESULTS

Expression of TGFα mRNA is primarily decidual and not embryonic. To determine whether expression of TGFα mRNA is primarily embryonic or maternal, we performed Northern blot analyses with poly(A)$^+$ RNAs from the uterus, decidua, and embryo (for a detailed definition of these terms, see Materials and Methods). The nature of the dissections performed is shown in Fig. 1, which illustrates the isolation of the embryo from the surrounding decidua for samples obtained at days 8 (A and B) and 11 (C and D) of gestation. Because it was difficult to isolate cleanly sufficient quantities of embryonic RNA at earlier gestations, this comparison was limited to days 10, 11, and 14. Probing of the decidual and uterine poly(A)$^+$ RNAs from days 10 and 11 of gestation resulted in specific hybridization to a transcript of approximately 4.5 kilobases (kb) (Fig. 2). This transcript (which was identified under conditions of high stringency) comigrated with authentic TGFα mRNA isolated from retrovirus-transformed rat cells (data not shown). Since our previous studies had suggested the presence of a single TGFα gene in the rat genome, we conclude that the 4.5-kb transcript corresponds to TGFα mRNA. It is particularly significant, therefore, that the 4.5-kb transcript was absent from the day 10 and 11 embryonic poly(A)$^+$ RNAs. Similarly, although poly(A)$^+$ RNA from the day 14 uterus and, to a lesser extent, the placenta contained TGFα mRNA, the respective embryonic sample did not (Fig. 2). To exclude the possibility that the embryonic RNAs were degraded, we analyzed all poly(A)$^+$ RNAs by electrophoresis on agarose-urea gels. The results of these analyses demonstrated that the failure to detect TGFα transcripts in the embryonic samples was not the result of degradation (data not shown). Moreover, when the Northern blot shown in Fig. 2 was reprobed with a cloned cDNA encoding γ-actin, all poly(A)$^+$ RNAs yielded bands of similar intensities (data not shown). Finally, it should be noted that poly(A)$^+$ RNA from the day 14 uterus consistently contained an additional higher-molecular-weight transcript of approximately 10 kb (Fig. 2, lane G); the identity of this transcript has not been established.

To determine whether the apparent expression of TGFα mRNA in the uterus was due to contamination by the decidua, we performed Northern blot analyses of decidual and uterine poly(A)$^+$ RNAs at earlier gestational ages when the two tissues are more distinct. In addition, we examined those intersegmental regions of the uterus between the implantation sites that did not show evidence of a decidual reaction. The results (Fig. 3) indicated that at earlier times of gestation (i.e., days 7, 8, and 9), poly(A)$^+$ RNAs from the decidual samples contained significant levels of TGFα mRNA, whereas the uterine samples (including those from the day 7 and day 9 intersegmental regions) did not. In contrast, uterine samples from days 10, 11, and 14 (compare Fig. 2 and 3) contained significant levels of TGFα mRNA that had, by day 14, equaled those present in the day 11 decidual sample. We interpret these results to indicate that expression is predominantly decidual and that the appearance of TGFα mRNA in uterine samples at later gestational ages is due to resorption of the decidua by the uterus. This interpretation is strengthened by the results of our in situ hybridization analysis described below.

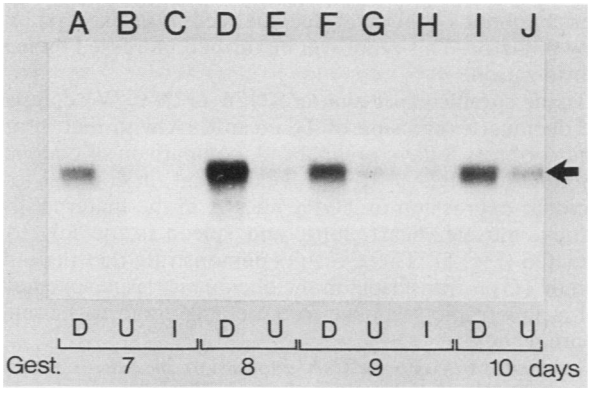

FIG. 3. Decidua-specific expression of TGFα mRNA. Northern analysis of poly(A)$^+$ RNA (10 μg per lane) from decidua (D, lanes A, D, F, and I), uterus (U, lanes B, E, G, and J), and intersegmental regions of the uterus (I, lanes C and H) at days 7 (lanes A to C), 8 (lanes D to E), 9 (lanes F to H), and 10 (I to J) of gestation (Gest.), probed with the cloned rat TGFα cDNA. The arrow indicates the migration of the 4.5-kb TGFα mRNA.

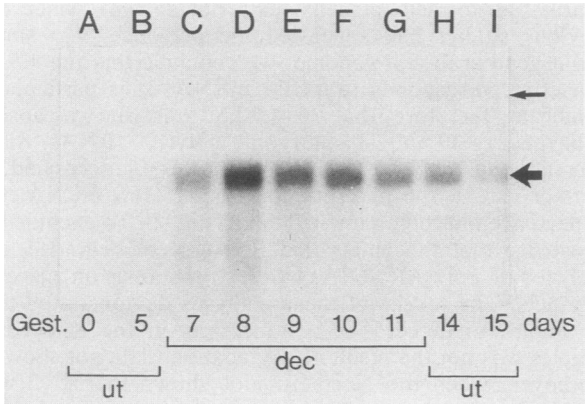

FIG. 4. Ontogeny of TGFα mRNA expression in the decidua. Northern analysis of poly(A)$^+$ RNAs (10 μg per lane) from uterus (ut, lanes A, B, H, and I) and decidua (dec, lanes C to G) at various gestational ages (Gest.), probed with the cloned rat TGFα cDNA. The large and small arrows indicate the migration of the 4.5-kb TGFα mRNA and an unidentified transcript of approximately 10 kb, respectively.

Ontogeny of decidual expression. Because the results presented in Fig. 3 suggested that the levels of decidual TGFα mRNA varied with the age of gestation, we examined the ontogeny of expression by contrasting Northern analyses of poly(A)$^+$ RNAs from day 7 through day 11 decidua. In addition, we also included, for comparison, uteri from nonpregnant animals and from pregnant animals at days 5, 14, and 15 of gestation. The latter were used because in nonpregnant and day 5 (preimplantation) uteri, the endometrium has not yet differentiated into decidua. Similarly, at days 14 and 15, only decidua basalis remains, and it is firmly attached to the uterus. The results (Fig. 4) show that significant expression of TGFα mRNA was observed only in the decidual samples and could be detected at day 7. The highest level of expression was observed with poly(A)$^+$ RNA from the day 8 decidua, with subsequent levels declining slowly through day 15. In contrast, the levels of this transcript in the nonpregnant and day 5 uteri were either low or undetectable. Because by this method it was impossible to examine specifically the decidual tissue at all stages of development, we cannot exclude the possibility that the uterus contains islands of decidual cells still expressing the growth factor. This point will be further addressed by in situ hybridization.

Tissue specific expression of TGFα mRNA. We compared the decidual expression of TGFα mRNA with that in other organs of rats 8 days pregnant. A comparison of equivalent amounts of poly(A)$^+$ RNA revealed that, in contrast to the decidua, expression of TGFα mRNA in the maternal liver, kidney, muscle, heart, lung, and spleen is too low to be detected (Fig. 5). These results demonstrate that the induction of TGFα transcripts in the decidua is tissue specific and not a pleiotropic response to the changing hormonal milieu of pregnancy.

Analysis of TGFα mRNA expression in situ. To further investigate the cellular sites of expression and, in particular, to address the uncertainties raised concerning uterine expression of TGFα mRNA, we performed in situ hybridization. Several probes were used in these analyses, including ^{32}P-labeled oligonucleotides and fragments of the cloned rat TGFα cDNA, as well as ^{3}H-labeled TGFα-specific RNA transcripts (cRNA). Although the background hybridization is significantly lower with the ^{3}H-labeled cRNA, the results obtained using these various probes are otherwise indistinguishable. Whereas there was very little hybridization of the TGFα cRNA to uterine tissue at day 5 of gestation (Fig. 6A and B), intense hybridization was observed with sections of the day 8 decidua (Fig. 6C and D). Moreover, this hybridization, which is diminished by day 11 (E and F), appears to be the direct result of hybridization to RNA since it can be abolished by prior treatment of day 8 decidual sections with RNase (G and H). Of particular significance is the finding that although there is marked hybridization to the day 8 decidual cells, there is little, if any, hybridization to the embryo and associated membranes (C and D).

Figure 7 shows, at higher magnification, the hybridization of the ^{3}H-labeled TGFα transcripts to day 8 decidual tissue (A and B). We have consistently observed that hybridization is not uniform over the entire decidua, but appears to be concentrated over those regions adjacent to the embryo (C and D). Figure 7 also includes a section which contains both day 8 uterus and decidua and clearly demonstrates that expression of TGFα mRNA is specific to the latter (E and F) at this gestational age. Indeed, we have consistently failed to observe significant hybridization to uterine tissue at any stage of gestation (data not shown).

As noted above, we failed to detect significant expression of TGFα mRNA in embryos by either Northern blotting or in situ hybridization. Figure 8 shows, for example, an in situ hybridization analysis of a day 11 embryo at low magnification and reveals the lack of specific hybridization. It is important to note that this section is representative of all three germ layers, none of which display significant hybridization over background. In addition, we also failed to detect significant hybridization to either embryo-associated membranes or the yolk sac. Since we have not exhaustively examined serial sections of embryos at every gestational age, we cannot exclude the possible localized expression of TGFα in the embryo at some point in development. However, our data clearly demonstrate that the marked expression of TGFα seen early in gestation occurs in the maternal decidua and not in the embryo.

DISCUSSION

We examined the expression of TGFα mRNa during rat development by both Northern blot analysis and in situ hybridization. Our findings demonstrate that the high levels of TGFα mRNA observed during early development are the

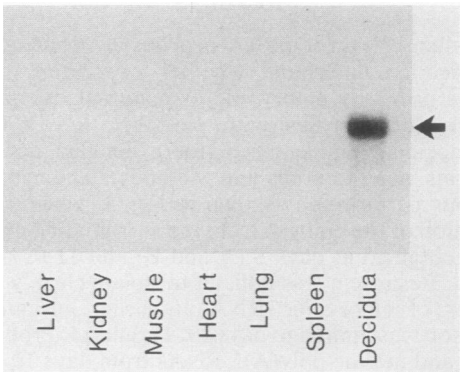

FIG. 5. Tissue-specific expression of TGFα mRNA. Northern analysis of poly(A)$^+$ RNAs (10 μg per lane) from various tissues of a pregnant rat at day 8 of gestation. The arrow indicates the migration of the 4.5-kb TGFα mRNA.

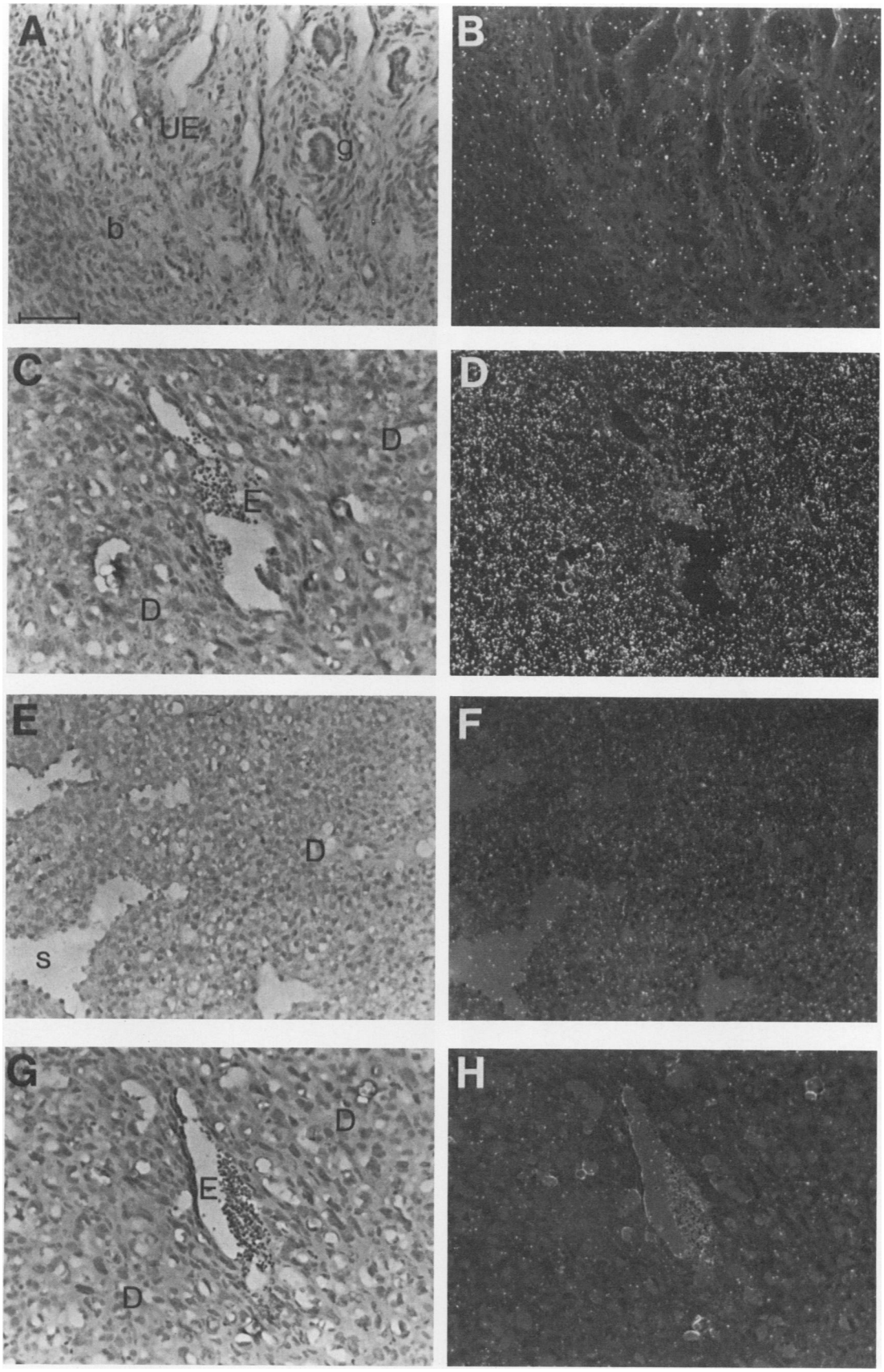

FIG. 6. In situ localization of TGFα mRNA expression. Bright (A, C, E, and G)- and dark (B, D, F, and H)-field photomicrographs of maternal and embryonic tissues probed with ^{3}H-labeled TGFα cRNA. (A and B) Uterus (UE) from day 5 of gestation (preimplantation) showing no hybridization to either the basal layer (b) or endometrial glands (g). (C and D) Decidua (D) with cross section of the embryonic tube (E) from day 8 of gestation showing intense hybridization to the stromal cells of the decidua. (E and F) Decidua from day 11 of gestation showing less hybridization than that observed with the day 8 decidua. s, Decidual sinusoid. (G and H) Section of day 8 decidua and embryonic tube, adjacent to that shown in panels B and C, treated with RNase (10 μg/ml) at 37°C for 30 min before hybridization. Magnification, ×20; bar = 50 μm.

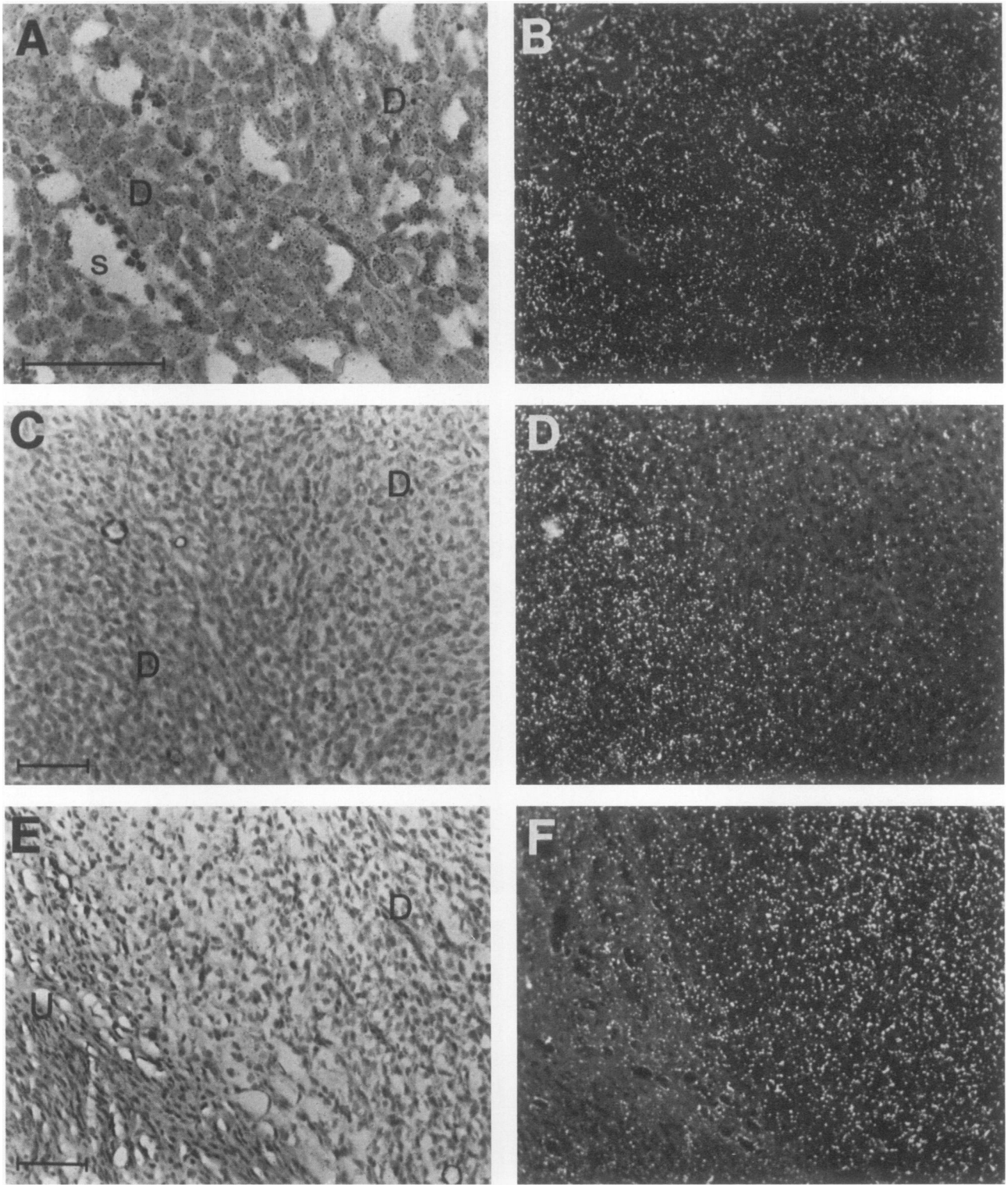

FIG. 7. In situ localization of TGFα mRNA expression. Bright (A, C, and E)- and dark (B, D, and F)-field photomicrographs of day 8 decidua (D) hybridized with ^{3}H-labeled TGFα cRNA. (A and B) Intense hybridization to stromal cells of the decidua. s, Decidual sinusoid. (C and D) Intensity of hybridization is greatest in that portion of the decidua closest to the embryo (lower left corner) and decreases with distance from the embryo. (E and F) Hybridization is observed in the decidua, not in the uterus (U). Magnification, ×40; bar = 100 μm.

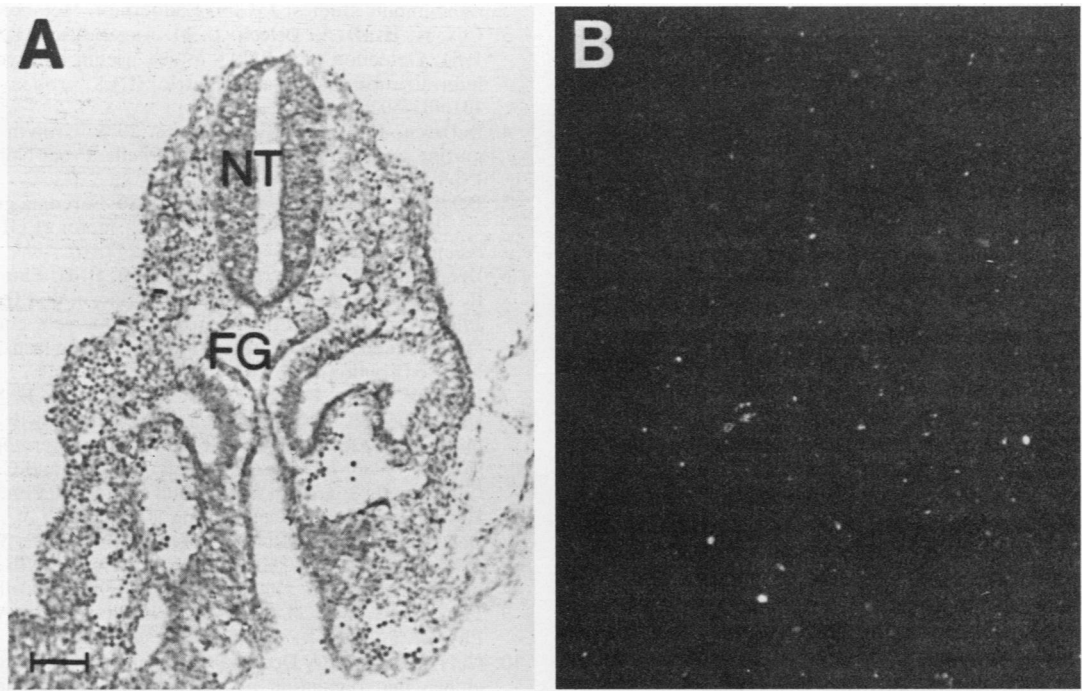

FIG. 8. In situ hybridization to section of rat embryo. Bright (A)- and dark (B)-field photomicrographs of a day 11 embryo hybridized in situ with a ^{32}P-labeled TGFα cDNA. The probe was a 700-base-pair *Sma*I fragment of the cloned TGFα cDNA that was nick translated as described in Materials and Methods. This fragment includes all of the coding region of the TGFα transcript. This section shows no specific hybridization to the in situ probe. NT, Neural tube; FG, foregut. Magnification, ×10.

result of expression in the maternal decidua and not, as previously suggested, in the embryo. This expression appears to be induced at day 6 after implanation of the embryo and is highest at day 8, when the embryo is undergoing gastrulation and neurulation. After this peak, the levels of TGFα mRNA slowly decline through day 15 as the decidua is being resorbed. Interestingly, the expression of TGFα transcript at day 8 is not uniform throughout the decidua but is highest in those regions immediately adjacent to the embryo. However, within these latter areas, the hybridization is uniform, and the vast majority of stromal cells appear to express TGFα mRNA. Finally, the decidual expression of TGFα mRNA is a tissue-specific response, and transcripts cannot be detected in other maternal tissues.

That TGRα is expressed in the developing rodent embryo has been suggested by previous studies. Nexo et al. (21) found that mouse embryos between days 11.5 and 17.5 of gestation contained more EGF receptor-binding activity than could be accounted for by an EGF-specific radioimmunoassay. These data were interpreted to suggest that the additional EGF receptor-competing activity resulted from the embryonic expression of TGFα. Twardzik et al. (32, 33) subsequently reported a high level of TGFα in mouse embryos as detected by both radioreceptor competition and bioassay. Since this activity was highest at day 7 of gestation, fell sharply to low levels at days 10 and 11, and increased to an minor peak at day 13, the ontogeny of expression differed from that reported by Nexo et al. (21). The highest levels of TGFα protein were reported to approach those produced by cultured retrovirus-transformed cells. Finally, Lee et al. (14) reported the expression of high levels of TGFα mRNA during rat development; the peak of expression occurred at day 9 of gestation, with the levels becoming nondetectable between days 11 and 13. Since the coital plug dates were defined by Twardzik et al. (32, 33) and Lee et al. (14) as day 0 and day 1, respectively, and rat development is delayed relative to that in the mouse by approximately 1 day, the major peaks of mouse TGFα polypeptide and rat TGFα mRNA appeared to coincide.

The results presented here further support previous claims of expression of TGFα during rodent development. Moreover, the ontogeny of expression with respect to the major peak (rat day 8, plug date = day 0) is in agreement with the earlier results of both Lee et al. (14) (rat day 9, plug date = day 1) and Twardzik (32) (mouse day 7, plug date = day 0). However, the present findings clearly reveal a different site of expression than was implied by the earlier studies. In addition, our results also indicate that the levels of TGFα mRNA do not decline as rapidly at the later gestational ages as was previously suggested. We attribute these discrepancies to maternal contamination of the early gestation embryos in the previous studies. This possibility, acknowledged by Twardzik (32), would not only explain the contradiction with respect to the site of expression, but would also account for the apparently rapid decline in the expression of both mRNA and peptide seen previously. However, because the studies of Nexo et al. (21) and Twardzik et al. (32, 33) were based on the measurement of TGFα protein and not mRNA, we cannot exclude the possibility that TGFα synthesized in the decidua is localized in the embryo via maternal or transplacental transport. It is also important to point out that since we did not exhaustively section embryos at later gestational ages, our results do not exclude the localized expression of low levels of TGFα mRNA in the embryo at some stage in development. Our results do, however, clearly demonstrate that the very significant

expression of TGFα mRNA and, presumably, protein seen early in development (i.e., day 8 in rat) is of maternal and not embryonic origin.

The expression of TGFα in the decidua raises the interesting question as to its possible role. Formation of the decidua (decidualization) is a poorly understood phenomenon involving cell proliferation and migration that is coordinated with cellular growth in the uterine epithelium (for a general description, see reference 9). It apparently results from the differentiation and proliferation of stem cells in the endometrial stroma that may have originally derived from the bone marrow (13). Once the uterus is sensitized by appropriate hormones, decidualization is then triggered, in part, by implantation of the embryo. Since some decidual cells appear to contain EGF receptor (2, 23), it seems most likely that TGFα acts to induce, or amplify, the massive proliferation of these cells through a paracrine or autocrine mechanism. This interpretation is consistent with the findings that maximal DNA synthesis in the mouse decidua occurs at day 7 of gestation (corresponding to day 8 in rat) and that the zone of proliferation appears to radiate out from the implantation site (35). In addition, since one report claims that TGFα has angiogenic activity (29), it may play a role in promoting the extensive vascularization required for the decidualization reaction (10). Last, decidual cells secrete a prolactinlike protein (11, 24), and since EGF can apparently induce prolactin synthesis in the GH4 rat pituitary cell line (20), it is possible that TGFα regulates hormone production in this tissue.

In addition to its postulated role in the decidual reaction, it is also possible that TGFα produced in the decidua acts on the placenta and the embryo, both of which have been shown to contain significant levels of EGF receptor (2, 21, 23). Endocrine action on the embryo would presumably require that decidual TGFα be transported either via the maternal circulation or by an as yet unknown transplacental mechanism. In this regard, it may be interesting to note that TGFα has been detected in the urine of pregnant women (30). Finally, because mature rat TGFα is cleaved from the 159-amino acid transmembrane protein that may have distinct biological activities, we cannot exclude the possibility that the TGFα expressed in this developmental context is not processed and does not function as a growth factor.

The developmental expression of TGFα indicates a normal physiological function for this growth factor and shows that high levels of expression do not necessarily lead to a neoplastic response. Understanding the role of TGFα in this developmental context will ultimately require both an in situ localization of its site of action and an analysis of its effects on maternal and embryonic cells in vitro.

ACKNOWLEDGMENTS

We thank Joe D'Ercole, Shelly Earp, and Noreen Luetteke for a critical reading of the manuscript and Andy Blasband for teaching D.C.L. computer competency.

V.K.M.H. was the recipient of a research fellowship from the Medical Research Council of Canada. This work was supported by Public Health Service grant CA43793 to D.C.L. from the National Institutes of Health.

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