MOLECULAR AND CELLULAR BIOLOGY, Aug. 1988, p. 3415–3422
0270-7306/88/083415-08$02.00/0

Vol. 8, No. 8

Developmental Expression of Transforming Growth Factors Alpha and Beta in Mouse Fetus

JOSIAH N. WILCOX* AND RIK DERYNCK

Department of Molecular Biology, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080

Received 14 December 1987/Accepted 29 April 1988

Expression of mRNA for transforming growth factor alpha (TGF-α) and TGF-β1 during the fetal development of mice was evaluated by in situ hybridization. TGF-α mRNA was detected in 9- and 10-day fetuses but was absent in older fetuses. TGF-α mRNA-containing cells were found in the placenta, otic vesicle, oral cavity, pharyngeal pouch, first and second branchial arches, and developing kidneys. mRNA for TGF-β1 was present in hematopoietic cells of blood islands and capillaries and in the liver as it began to bud off on day 10 and function as a hematopoietic organ.

Two different proteins have been named transforming growth factors (TGFs). TGF-α is encoded as part of a 160-amino-acid transmembrane precursor (2, 12) and shares structural homology with epidermal growth factor (EGF) (12, 19). Both EGF and TGF-α bind to the same cell surface receptor and exert a mitogenic effect upon cells (28). TGF-β1 is a homodimeric polypeptide consisting of two 112-amino-acid monomers which are each derived from a 390-amino-acid secreted precursor (10–12). TGF-β is structurally unrelated to TGF-α and binds to separate receptors (4). TGF-β1 can be mitogenic for fibroblasts but inhibits proliferation of a variety of cell lines. Whether TGF-β stimulates or inhibits cell proliferation depends upon the growth conditions of the cells and the cellular physiology (23, 29, 31).

TGF-β1 is secreted by a very wide variety of normal and transformed cells in culture, and it is likely that all cultured cells display some level of TGF-β synthesis (9, 29). Little is known about tissue-specific synthesis of TGF-β1 in vivo. TGF-α, on the other hand, is secreted by retrovirus-transformed fibroblasts (30) and a wide variety of tumor cells derived from carcinomas or sarcomas (8). In addition, TGF-α is synthesized by normal skin keratinocytes (5) and normal brain cells (J. N. Wilcox and R. Derynck, J. Neurosci., in press). It has also been documented that TGF-α is transiently expressed during fetal development. The highest levels of TGF-α were found in 7- to 9-day-old mouse fetuses (32). Similar results have been obtained with rat embryos, in which the highest levels of TGF-α mRNA were present at days 8 to 9 and declined to nondetectable levels at day 13 (17). Because of the difficulty of separating early embryos from the placenta or other maternal tissue, it is important to determine the sites of TGF-α synthesis in more detail. This can be achieved by in situ hybridization histochemistry. Han et al. (14) recently showed by in situ hybridization and Northern (RNA) hybridization that the transient peak of TGF-α expression during embryonic development is localized in the maternal decidua. Since they were unable to find TGF-α expression in embryos, it was concluded that the TGF-α expression which was previously detected during fetal development by Northern analysis was from the maternal decidua and not from the embryo.

We report here a study by in situ hybridization of the developmental expression of TGF-α and TGF-β1. We confirmed that TGF-α is transiently expressed during fetal development. Whereas TGF-α expression was found in the placenta, TGF-α mRNA was also detected in several developing fetal organs, especially the otic vesicle, oral cavity, pharyngeal pouch, and kidneys. We also detected expression of TGF-β1, which predominantly took place in the hematopoietic cells of developing fetuses.

MATERIALS AND METHODS

Animals and tissues. CD1 female mice were obtained from Charles River Laboratory and housed with males until vaginal plugs were detected. The gestational age was determined from plug dates (the plug date is defined as day 0 of gestation). Animals were sacrificed at 9, 10, 12, 14, or 16 days of pregnancy by cervical dislocation, and the embryos were dissected from the uteri. Embryos and placentas were examined by in situ hybridization at all stages studied. The entire uterus containing two or more decidua was taken in some day 9 samples, and sections of the embryo and surrounding uterus were hybridized together.

The tissues were dissected in sterile phosphate-buffered saline (PBS) with the aid of a dissection microscope. The embryos were then fixed for 3 h in 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4) at 4°C, followed by immersion in 15% sucrose–PBS overnight at 4°C as a cryoprotectant. The embryos were sectioned in the sagittal plane at a 10-μm thickness on a cryostat and then mounted and immediately refrozen on gelatin-coated microscope slides. The sections were stored at −70°C with desiccant for up to 2 months before hybridization. There was no loss of hybridization signal over this period.

Hybridization probes. The murine TGF-α hybridization probe was a 1,400-base-pair genomic *Sau*3A fragment corresponding to a segment in exon 6 of the TGF-α gene. This segment in the 3′-untranslated region has its most upstream residue 6 bases 3′ to the stop codon. The *Sau*3A fragment was subcloned in the *Bam*HI site of plasmid sp64 in the antisense orientation with respect to the transcriptional direction from the sp6 promoter (R. Derynck, unpublished data). This probe has been used in Northern blots and recognizes a 5.0-kilobase TGF-α mRNA species in *ras*-transformed mammary epithelial cells (27). The TGF-β1 probe was the *Eco*RI fragment of pMurβ2 (10), corresponding to a cDNA containing the entire coding sequence for the TGF-β precursor. This 1,600-base-pair fragment was in-

* Corresponding author.

serted into the *Eco*RI site of plasmid sp65 in the antisense orientation with respect to the transcriptional direction from the sp6 promoter.

The probes were labeled with [α-^{35}S]ATP by transcription of linearized plasmids with sp6 RNA polymerase in a modification of the procedure of Melton et al. (22). The transcription mixture contained a 500 μM final concentration of each of the nucleotides CTP, GTP, and UTP and 12 μM [^{35}S]ATP (650 Ci/mmol; SA; Amersham Corp.), 40 U of RNasin (Promega Biotech), 2 mM spermidine, 10 mM dithiothreitol, 10 mM NaCl, and 40 mM Tris (pH 7.5) and was initiated by addition of 15 U of sp6 RNA polymerase (Promega). The reaction proceeded for 2 h at 37°C, followed by DNase digestion of the template, phenol-chloroform extraction, and ethanol precipitation. The specific activity of these probes was 162.5 Ci/mmol (about 10^9 cpm/μg).

In situ hybridization. In situ hybridizations were performed as described previously (7, 20), except for some slight modifications. The sections were removed from the freezer and immediately fixed for 10 min in 4% paraformaldehyde, rinsed for 5 min in 0.5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and immersed in a solution of 5 μg of proteinase K per ml in water for 10 min at room temperature. The sections were then washed with 0.5× SSC for 10 min, the slides around the sections were dried with a Kimwipe napkin, and 50 μl of hybridization buffer was applied without the probe (0.3 M NaCl, 20 mM Tris [pH 8.0], 5 mM EDTA, 1× Denhardt solution, 10% dextran sulfate, 10 mM dithiothreitol), and the sections were incubated at 42°C for 2 h in boxes containing filter paper saturated with 4× SSC and 50% formamide. The hybridization was initiated by adding 100,000 cpm of the probe in an additional 10 μl of hybridization buffer with 15 μg of *Saccharomyces cerevisiae* tRNA and allowed to proceed overnight at 50°C. At the end of the hybridization, the sections were washed twice for 10 min each time in 2× SSC, immersed in a solution of 20 μg of RNase A per ml in 0.5 M NaCl for 30 min at room temperature, and washed twice for 10 min in 2× SSC, followed by a high-stringency wash in 0.1× SSC at 42°C. All SSC solutions up to this point contained 10 mM β-mercaptoethanol and 1 mM EDTA to help prevent nonspecific binding of the probe. The tissue was then washed twice for 10 min each time in 0.5× SSC without β-mercaptoethanol and dehydrated by immersion in graded alcohols containing 0.3 M ammonium acetate. The sections were dried and coated with NTB2 nuclear emulsion (Eastman Kodak Co.) and exposed in the dark with desiccant at 4°C for 4 to 8 weeks. After development, the sections were counterstained with hematoxylin and eosin.

RESULTS

Mouse embryos were taken at days 9, 10, 12, 14, and 16 of pregnancy and screened for expression of TGF-α and TGF-β1 mRNAs by in situ hybridization. Young embryos (embryonic day 9, 10, or 12) were screened every 50 μm, and older embryos (day 14 or 16) were screened every 100 μm. Serial sections were hybridized to TGF-α or TGF-β1 ^{35}S-labeled riboprobes to allow direct comparison of the distribution of cells making each gene product. Since the TGF-α and TGF-β1 hybridizations were conducted in parallel with probes of equal specific activities, each can be considered the control for the other. Each probe labeled a distinct population of cells with no overlap in the distribution of hybridizing cells (Fig. 1). These results argue in favor of the specificity of the hybridization reaction and demonstrate that TGF-α and TGF-β1 mRNAs were not present at detectable levels in the same cell in mouse embryos at the times we studied.

TGF-α gene expression. TGF-α gene expression was confined to days 9 and 10 of embryogenesis. On day 9, hybridization was seen in both the placenta and embryo. TGF-α mRNA-containing cells were found in the syncytiotrophoblast layer of the placenta in a narrow border of cells adjacent to the maternal placenta (Fig. 2A). No TGF-α-positive cells were detected in placentas of older embryos (days 10, 12, and 14). This result is consistent with the earlier report of TGF-α expression in the placenta (14).

In contrast to a previously published report (14), positive hybridization of the TGF-α probe was seen in embryos on days 9 and 10. TGF-α mRNA-containing cells were found in the otic vesicle, oral cavity, pharyngeal pouch, first and second branchial arches, and developing mesonephric tubules of embryonic mouse kidneys (Fig. 2). TGF-α gene expression continued into day 10 in the otic vesicle and pharyngeal pouch but at much lower levels (data not shown). No TGF-α-hybridizing cells were detected after day 10. TGF-α gene expression at this stage of development, from day 12 onward, may be greatly reduced or nonexistent, although it is possible that there was still a low level of TGF-α gene expression that we could not detect because of limitations in the sensitivity of the technique.

TGF-β gene expression. TGF-β1-expressing cells in embryos had a much wider distribution than TGF-α cells and were found scattered throughout the blood islands and capillaries of day 9 to 10 embryos (Fig. 3A). These were small mononuclear cells with very little cytoplasm and appeared to be erythroid in origin. The cells in the blood islands which were positive for TGF-β1 mRNA hybridization were similar in appearance to other erythroid cells in these structures. Hybridizing cells in circulation were also seen in the chambers of developing hearts. The positive heart cells also seemed to be erythroid and were similar in appearance to the blood island cells. The only exception was a population of TGF-β1-positive cells that appeared to be structural in nature and were found as the innermost layer of cells in developing ventricles (Fig. 3B). It is possible that these cells derive from circulating erythroid cells, or they may arise independently.

Many cells positive for TGF-β1 mRNA hybridization were also found scattered throughout the embryos in many seemingly unrelated structures. These cells tended to be aligned in a single layer threading through a structure (Fig. 3C) and had the same morphology as that seen previously in the blood islands and hearts. Further examination suggested that these cells were probably in small capillaries. To support our hypothesis that these cells were erythroid, we took advantage of the fact that blood cells have endogenous peroxidase. Very often the endogenous peroxidase of blood cells causes problems with immunocytochemistry when diaminobenzidine and peroxide are used as a substrate. In this case, we took advantage of this fact and found that we were able to stain peroxidase-containing erythroid cells by immersing the tissue sections in a solution of diaminobenzidine and hydrogen peroxide (0.6 mg of diaminobenzidine per ml and 0.15% H_2O_2 in PBS). Staining for endogenous peroxidase on sections near those used for in situ hybridization indicated that the cells which contained detectable levels of TGF-β1 mRNA also contained endogenous peroxidase, thus supporting the hypothesis that the positive TGF-β cells were erythroid in nature.

Hybridization to cells in the blood islands and capillaries

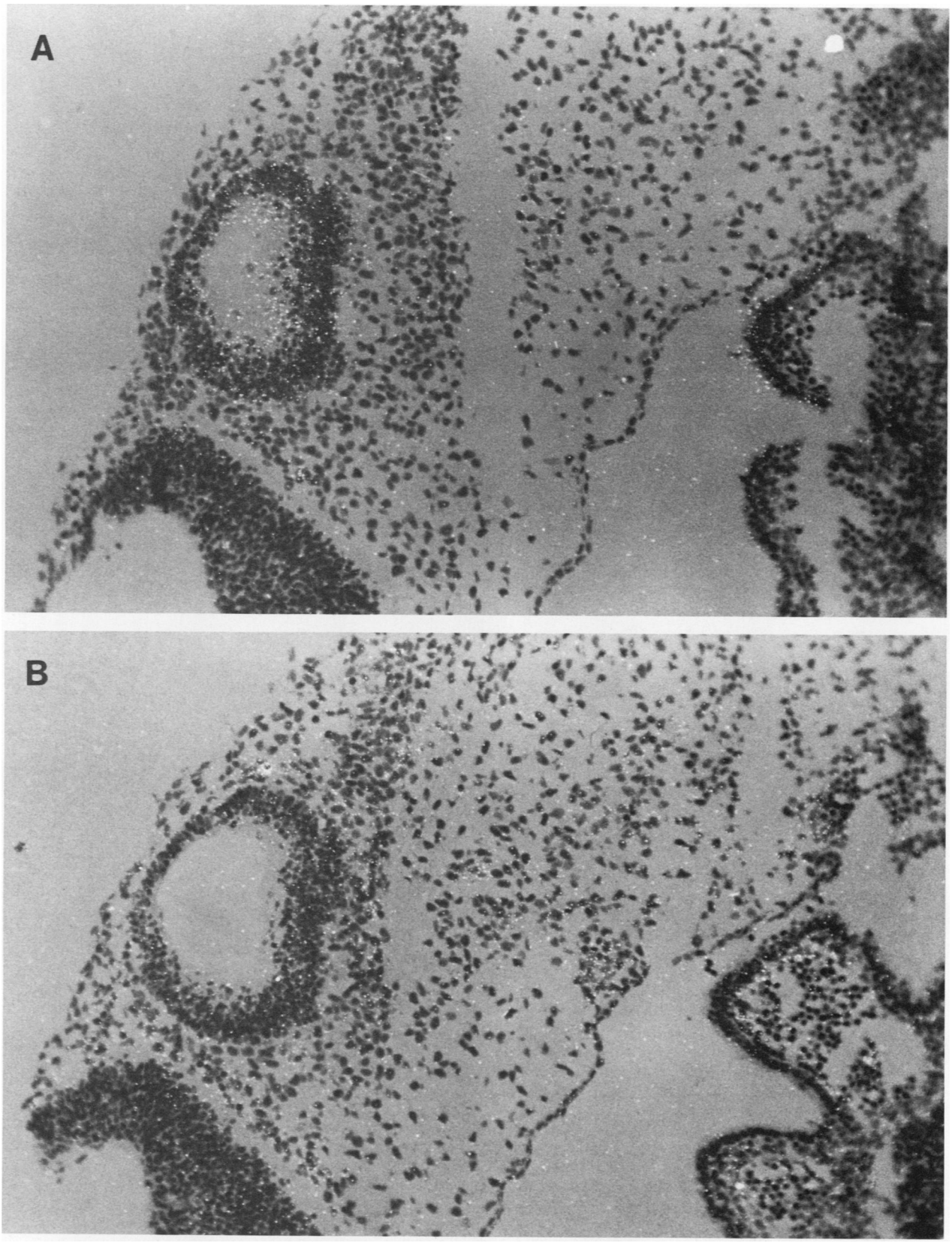

FIG. 1. In situ hybridization showing localization of TGF-α (A) and TGF-β (B) mRNAs in serial sections of a day 9 mouse embryo. Serial 10-μm sections were hybridized in parallel with ^{35}S-labeled riboprobes and exposed for 4 weeks. Note the different localization of each probe. TGF-α-positive cells were seen in the otic vesicle and luminal cells of the oral cavity (A), whereas after hybridization with TGF-β these cells were negative (B) and only scattered cells hybridized to the probe. Exposure time, 4 weeks. Magnification, 125×.

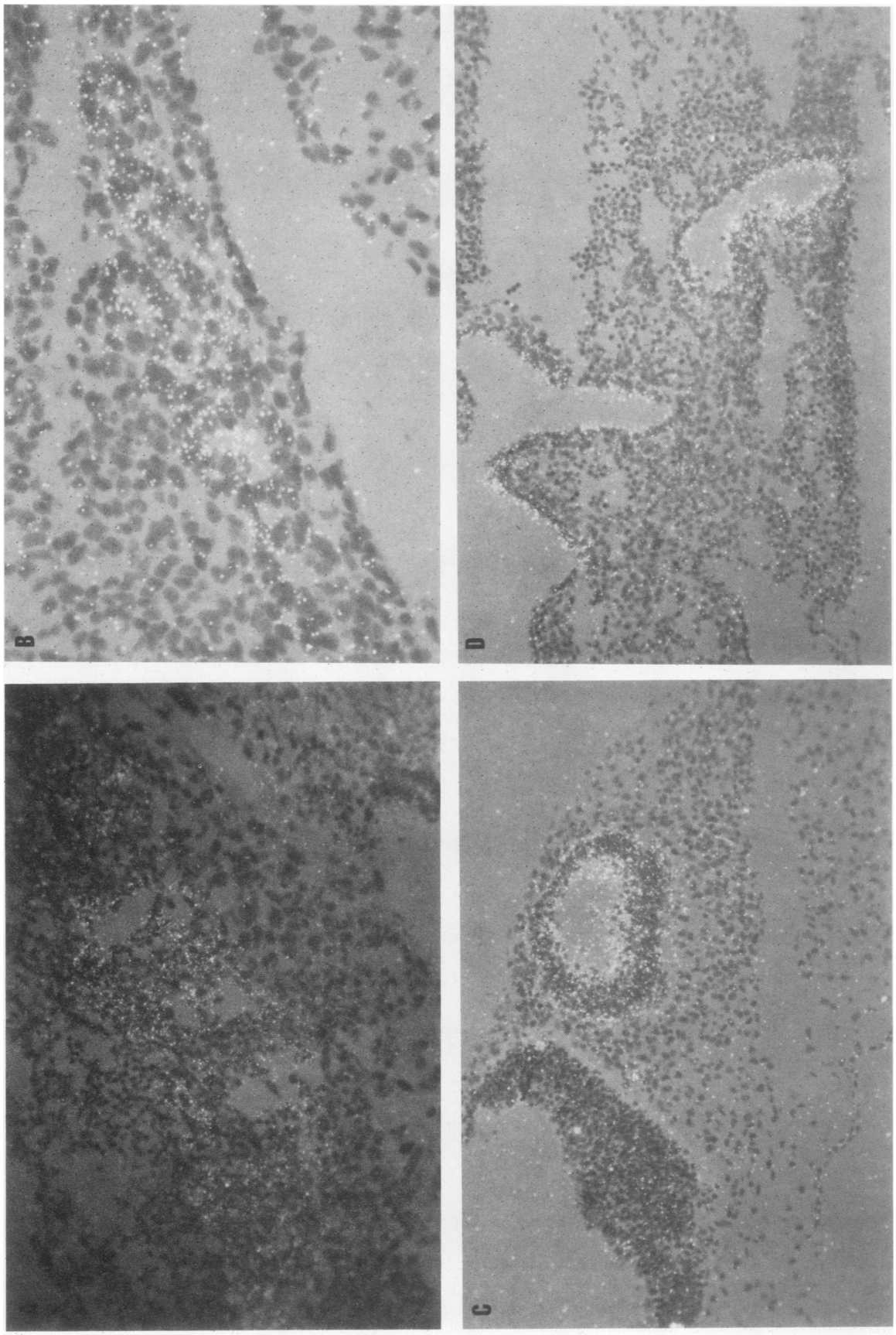

FIG. 2. Localization of TGFα-expressing cells by in situ hybridization in a 9-day-old mouse embryo. TGF-α mRNA-containing cells were found in the syncytiotrophoblast layer of the placenta (A [2-week exposure; magnification, ×125]), developing mesonephric tubules (B [4-week exposure; magnification, ×310]), otic vesicle (C [4-week exposure; magnification, ×125]), and cells lining the oral cavity and pharyngeal pouch (D [4-week exposure; magnification, ×125]).

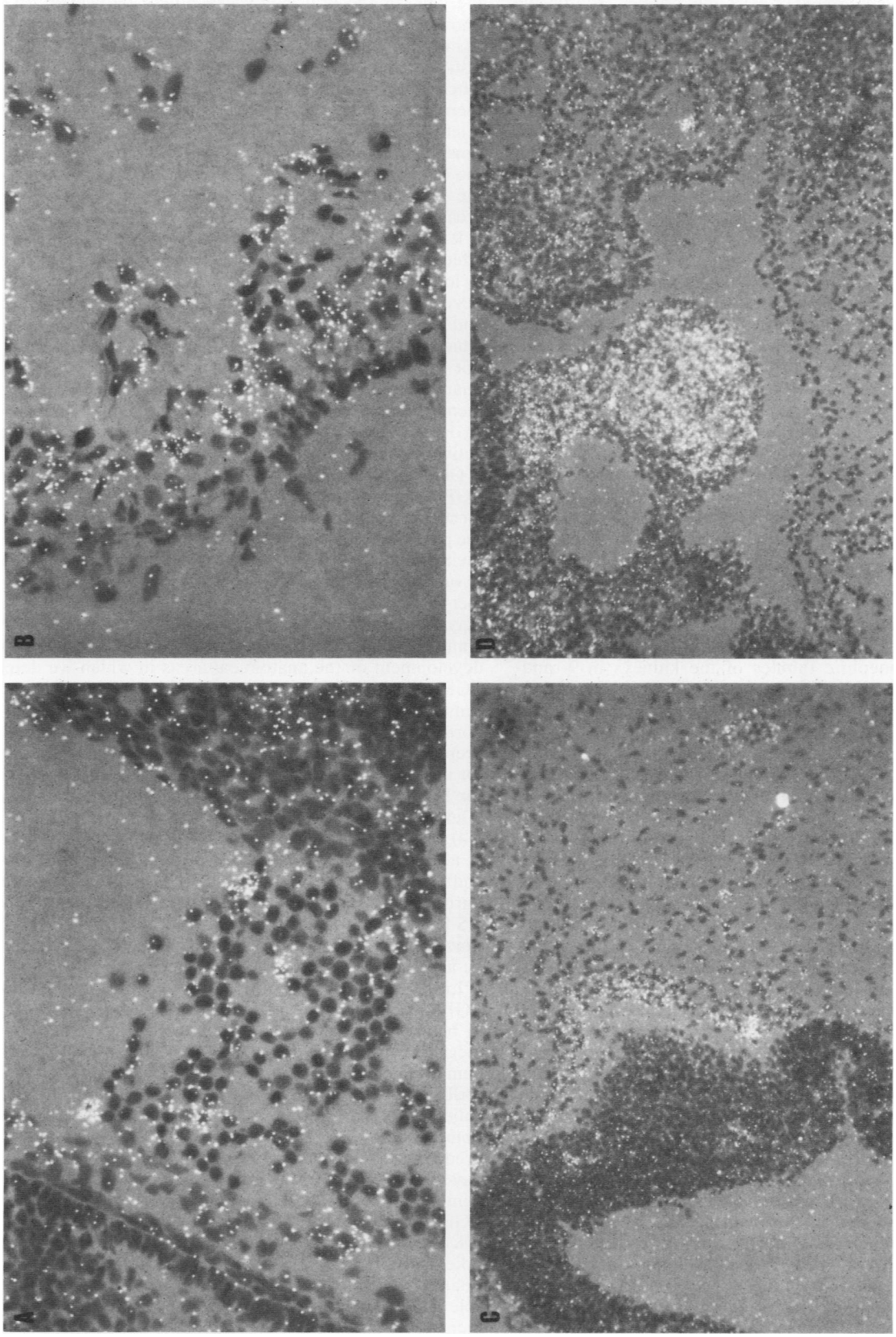

FIG. 3. Localization of TGF-β1-expressing cells by in situ hybridization in a 9- or 10-day-old mouse embryo. TGF-β1 mRNA-containing cells were found in scattered erythroid cells of the aortic blood island (A [magnification, ×310]). Similar cells were found scattered throughout the embryo, most often aligned in the fetal capillaries (C [magnification, ×125]). TGF-β1-positive cells were also found embedded in the wall of the heart (B [magnification, ×310]). Beginning on day 10, the fetal liver is a major site of TGF-β1 synthesis (D [magnification, ×125]). Exposure time, 4 weeks.

was not seen after day 12. Beginning on day 10 and continuing through day 14, the liver was the primary source of TGF-β1 mRNA synthesis in embryos (Fig. 3D). The liver first buds off and begins developing on day 10. At this stage, it appeared that most of the cells in the liver synthesized TGF-β1 mRNA. By day 14, fewer liver cells contained TGF-β1 mRNA and those that did seemed to be clustered in islands of expressing cells. No hybridization to TGF-β cRNA was detected in liver cells on day 16 of fetal development.

DISCUSSION

We have shown the localization of TGF-α and -β gene expression in mouse embryos by using in situ hybridization. It has previously been demonstrated that there is transient TGF-α expression in rodent embryos. Nexo et al. (24) demonstrated that there was EGF-like activity in mouse embryos between days 11.5 and 17.5 of gestation. It was subsequently shown that TGF-α-like peptides are present in mouse embryos, peak at days 12 to 13, and then decline to a nondetectable level at later stages of development (32). These data were further supported by Lee et al. (17), who showed the presence of TGF-α in 8- to 10-day-old rat embryos by Northern blot hybridization. However, it was recently reported that TGF-α mRNA at this stage of embryonic development could be detected only in the maternal decidua and not in developing fetuses (14). We have now shown by in situ hybridization that TGF-α is present not only in the placenta, where it is apparently located in the fetal compartment, but also in the otic vesicle, oral cavity, pharyngeal pouch, first and second branchial arch, and developing mesonephric tubules of the kidneys in 9- and 10-day-old mouse fetuses. In addition, we have shown that TGF-β1 is expressed during fetal development, especially in differentiating hematopoietic cells.

The detection of TGF-α expression in the placenta and several distinct loci in fetuses raises the question of the role of this peptide growth factor during development. TGF-α exerts its mitogenic effect through the EGF receptors at the cell surface. These receptors are expressed in mouse embryos as early as day 11 and continue to be detected through day 17 of gestation (24). It is thus likely that TGF-α can display a mitogenic effect on several cell populations in an autocrine, paracrine, or endocrine fashion and may represent a fetal analog of EGF. The presence of TGF-α in the oral cavity and the pharyngeal pouch suggests that it plays a role in the development of tissues in this area. This would be consistent with the findings of Pratt (25), who showed the mitogenic effect of EGF and TGF-α on the epithelium of the palate. These investigators suggested that TGF-α expressed by epithelial cells in the palate at about day 12 of gestation acts in an autocrine fashion to support the rapid growth of these cells during palatal development. It has also been shown that TGF-α is consistently expressed in squamous carcinomas which are of epithelial origin (8). In addition, TGF-α expression has recently been detected in normal human keratinocytes, in which TGF-α can induce its own expression via an autocrine mechanism (5). The localization of TGF-α synthesis in the oral cavity and pharyngeal pouch and the evidence that TGF-α could act via an autocrine mechanism, not only in tumor cells but also in normal epithelial cells, support the proposed role of TGF-α in palatal development. It is important in this context that TGF-α apparently induces a more pronounced mitogenic effect upon skin keratinocytes than does EGF (1).

It is not known whether there is any TGF-α synthesis in the cells of the oral cavities of adults. However, EGF is known to be synthesized in high levels by the salivary glands of mice (6), which are derived from the oral cavity. We have also detected transient expression of TGF-α in fetal kidneys, whereas conflicting data (14, 18) make it unclear whether there is any TGF-α expression in adult kidneys. In contrast, relatively high TGF-α mRNA levels have been detected in renal carcinoma cells (8). Finally, the synthesis of TGF-α in the otic vesicle, which is in part formed as an extension of the brain, may correlate with TGF-α expression in some areas of adult brains (Wilcox and Derynck, in press).

It is generally assumed that exertion of a direct mitogenic effect is the major activity of EGF and TGF-α. Therefore, it is logical to postulate a mitogenic role for TGF-α during development. It is, however, important to realize that EGF and TGF-α can exert a variety of cellular effects, several of which are unrelated to mitogenicity (1, 3). In addition, it is not known whether TGF-α synthesis is accompanied by quantitative proteolytic cleavage of TGF-α from the transmembrane precursor (2). It is conceivable that the uncleaved TGF-α precursor at the cell surface exerts distinct biological activities.

Our results confirm the recently reported detection of TGF-α in the placenta by Han et al. (14), but we have localized the expression in cells of the fetal compartment of the placenta. However, our results also clearly disagree with their findings, since they detected no TGF-α mRNA in fetal tissue, whereas we localized TGF-α mRNA levels at discreet loci in 9- to 10-day-old fetuses. Several technical reasons may account for this discrepancy. It is not clear whether Han et al. (14) examined specifically the same stages of fetal development or the anatomical areas in which we detected TGF-α mRNA. Furthermore, there could be a difference in sensitivity between their methodology and ours. They reported that the RNA used for Northern hybridization was prepared from fresh frozen tissues, whereas the fixed tissues for in situ hybridization were in PBS or sucrose-PBS at 4°C for 2 days. Significant RNA degradation in tissues can occur after 1 day at 4°C, even in paraformaldehyde-fixed tissues (34). Another difference in methodology is the fact that Han et al. (14) used ^{3}H-labeled cRNAs as hybridization probes and exposed the hybridized sections for autoradiography during 7 to 21 days, whereas this report describes the use of ^{35}S-labeled cRNA probes and autoradiographic exposure times of 4 to 8 weeks. It is likely that higher sensitivity can be achieved by using the latter conditions.

Little is known about developmental expression of TGF-β. Immunocytochemical analysis has localized TGF-β in bovine liver and bone. Immunoreactivity was found in megakaryocytes and mononuclear cells in bone marrow, hematopoietic stem cells in fetal liver, and the thymus, as well as a population of epithelial cells lining the kidney calices (13). The results of our study of murine fetal development are in agreement with these findings and show predominant TGF-β1 expression in hematopoietic cells. On day 9, the circulating blood cells function as the predominant hematopoietic organ. At this time, we found expression of TGF-β1 in hematopoietic cells of blood islands and capillaries. As the liver begins to bud off on day 10, it is hematopoietic in its earliest stages of development and shows relatively high levels of TGF-β1 mRNA. It is unclear what role TGF-β1 synthesis plays in the function and development of hematopoietic cells. It has previously been shown that mitogenic stimulation of T lymphocytes results in a significant increase of TGF-β1 mRNA (16). However, acid-

activated TGF-β1 clearly displays growth-inhibitory activation for lymphocytes and other hematopoietic cells (15, 16, 26). In addition, TGF-β1 is a potent chemoattractant for monocytes and can induce the synthesis of mediators of fibroblast growth by monocytes (33).

The developmental expression of TGF-α and TGF-β1 strongly suggests that both peptides play a physiological function during fetal development. Our understanding of these functions is, however, minimal and will require extensive analysis of the possible functions of TGF-α and TGF-β1 in vitro and in vivo.

ADDENDUM IN PROOF

Heine et al. (U. I. Heine, E. F. Munoz, K. C. Flanders, L. R. Ellingsworth, H.-Y. Peter-Lam, N. L. Thompson, A. B. Roberts, and M. B. Sporn, J. Cell Biol. **105**:2861–2876, 1987) have recently demonstrated the localization of TGF-β in 11- to 18-day-old mouse fetuses. Discrepancies between our TGF-β results and the results of Heine et al. could be due to differences in the stages of embryonic development and in the detection methodology. The in situ hybridization data are specific for TGF-β1 mRNA, whereas the immunochemistry utilized an antiserum that likely recognized different TGF-β species.

LITERATURE CITED

1. **Barrandon, Y., and H. Green.** 1987. Cell migration is essential for sustained growth of keratinocyte colonies: the roles of transforming growth factor-α and epidermal growth factor. Cell **50**:1131–1137.
2. **Bringman, T. S., P. B. Lindquist, and R. Derynck.** 1987. Different transforming growth factor-α species are derived from a glycosylated and palmitoylated transmembrane precursor. Cell **48**:429–440.
3. **Carpenter, G.** 1979. Epidermal growth factor. Annu. Rev. Biochem. **48**:193–216.
4. **Cheifetz, S., J. A. Weatherbee, M. L.-S. Tsang, J. K. Anderson, J. E. Mole, R. Lucas, and J. Massagué.** 1987. The transforming growth factor-β system, a complex pattern of cross-reactive ligands and receptors. Cell **48**:409–415.
5. **Coffey, R. J., R. Derynck, J. N. Wilcox, T. S. Bringman, A. S. Goustin, H. L. Moses, and M. R. Pittelkow.** 1987. Production and autoinduction of transforming growth factor-α in human keratinocytes. Nature (London) **328**:817–820.
6. **Cohen, S.** 1962. Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the newborn animal. J. Biol. Chem. **237**:1555–1562.
7. **Cox, K. H., D. V. De Leon, L. M. Angerer, and R. C. Angerer.** 1984. Detection of mRNAs in sea urchin embryos by *in situ* hybridization using asymmetric RNA probes. Dev. Biol. **101**: 485–502.
8. **Derynck, R., D. V. Goeddel, A. Ullrich, J. V. Gutterman, R. D. Williams, T. S. Bringman, and W. H. Berger.** 1987. Synthesis of mRNAs for transforming growth factors α and β and the epidermal growth factor receptor by human tumors. Cancer Res. **47**:707–712.
9. **Derynck, R., J. A. Jarrett, E.-Y. Chen, D. H. Eaton, J. R. Bell, R. K. Assoian, A. B. Roberts, M. B. Sporn, and D. V. Goeddel.** 1985. Human transforming growth factor-β cDNA sequence and expression in tumor cell lines. Nature (London) **316**:701–705.
10. **Derynck, R., J. A. Jarrett, E.-Y. Chen, and D. V. Goeddel.** 1986. The murine transforming growth factor-β precursor. J. Biol. Chem. **261**:4377–4379.
11. **Derynck, R., and L. Rhee.** 1987. Sequence of the porcine transforming growth factor-β precursor. Nucleic Acids Res. **15**: 3187.
12. **Derynck, R., A. B. Roberts, M. W. Winkler, E.-Y. Chen, and D. V. Goeddel.** 1984. Human transforming growth factor-α: precursor structure and expression in *E. coli*. Cell **38**:287–297.
13. **Ellingsworth, L.-R., J. E. Brennan, K. Fok, D. M. Rosen, H. Bentz, K. A. Piez, and S. M. Seyedin.** 1986. Antibodies to the N-terminal portion of cartilage-inducing factor A and transforming growth factor-β. J. Biol. Chem. **261**:12362–12369.
14. **Han, V. K. M., E. S. Hunter III, R. M. Pratt, J. C. Zendegui, and D. C. Lee.** 1987. Expression of rat transforming growth factor alpha mRNA during development occurs predominantly in the maternal decidua. Mol. Cell. Biol. **7**:2335–2343.
15. **Kehrl, J. H., A. B. Roberts, L. M. Wakefield, S. Jakowlew, M. B. Sporn, and A. S. Fauci.** 1986b. Transforming growth factor-β is an important immunomodulatory protein for human B lymphocytes. J. Immunol. **137**:3855–3860.
16. **Kehrl, J. H., L. M. Wakefield, A. B. Roberts, S. Jakowlew, M. Alvares-Mon, R. Derynck, M. B. Sporn, and A. S. Fauci.** 1986. The production of TGF-β by human T lymphocytes and its potential role in the regulation of T cell growth. J. Exp. Med. **163**:1037–1050.
17. **Lee, D. C., R. Rochford, G. J. Todaro, and L. P. Villareal.** 1985. Developmental expression of rat transforming growth factor-α mRNA. Mol. Cell. Biol. **5**:3644–3646.
18. **Lee, D. C., T. M. Rose, N. R. Webb, and G. J. Todaro.** 1985. Cloning and sequence analysis of a cDNA for rat transforming growth factor-α. Nature (London) **313**:489–491.
19. **Marquardt, H., M. W. Hunkapiller, L. E. Hood, and G. J. Todaro.** 1984. Rat transforming growth factor type 1 structure and relation to epidermal growth factor. Science **223**:1079–1082.
20. **Mason, A. J., S. L. Pitts, K. Nikolics, E. Szonyi, J. N. Wilcox, P. H. Seeburg, and T. A. Stewart.** 1986. The hypogonadal mouse: reproductive functions restored by gene therapy. Science **234**:1372–1378.
21. **Massagué, J.** 1983. Epidermal growth factor-like transforming growth factor II. Interaction with epidermal growth factor receptors in human plasma membranes on A431 cells. J. Biol. Chem. **258**:13614–13620.
22. **Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green.** 1984. Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage sp6 promoter. Nucleic Acids Res. **12**:7035–7056.
23. **Moses, H. L., R. F. Tucker, E. B. Leof, R. J. Coffey, J. Halper, and G. D. Shipley.** 1985. Type β transforming growth factor is a growth stimulator and a growth inhibitor, p. 65–71. *In* J. Feramisco, B. Ozanne, and C. Stiles (ed.), Cancer cells 3: growth factors and transformation. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Nexo, E., M. D. Hollenberg, A. Figueroa, and R. M. Pratt.** 1980. Detection of epidermal growth factor-urogastrone and its receptor during fetal mouse development. Proc. Natl. Acad. Sci. USA **77**:2782–2785.
25. **Pratt, R. M.** 1987. Role of epidermal growth factor in embryonic development. Curr. Top. Dev. Biol. **22**:175–192.
26. **Rook, A. H., J. H. Kehrl, L. M. Wakefield, A. B. Roberts, M. B. Sporn, D. B. Burlington, H. C. Lane, and A. S. Fauci.** 1986. Effects of transforming growth factor-β on the functions of natural killer cells: depressed cytolytic activity and blunting of interferon-responsiveness. J. Immunol. **136**:3916–3920.
27. **Salomon, D. S., I. Perroteau, W. R. Kidwell, J. Tam, and R. Derynck.** 1987. Loss of growth responsiveness to epidermal growth factor and enhanced production of α-transforming growth factor in ras-transformed mouse mammary epithelial cells. J. Cell. Physiol. **130**:297–304.
28. **Schreiber, A. B., M. E. Winkler, and R. Derynck.** 1986. Transforming growth factor-α is a more potent angiogenic mediator than epidermal growth factor. Science **232**:1250–1253.
29. **Sporn, M. B., A. B. Roberts, L. M. Wakefield, and B. de Crombrugghe.** 1987. Some recent advances in the chemistry and biology of transforming growth factor-β. J. Cell Biol. **105**:1039–1045.
30. **Todaro, G. J., D. C. Lee, N. R. Webb, T. M. Rose, and J. P. Brown.** 1985. Rat type-α transforming growth factor: structure and possible function as a membrane receptor, p. 51–58. *In* J.

Feramisco, B. Ozanne, and C. Stiles (ed.), Cancer cells 3: growth factors and transformation. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

31. **Tucker, R. F., G. D. Shipley, H. L. Moses, and R. W. Holley.** 1984. Growth inhibitor from BSC-1 cells closely related to type β transforming growth factor. Science **226**:705–707.
32. **Twardzik, D. R.** 1985. Differential expression of transforming growth factor-α during prenatal development of the mouse. Cancer Res. **45**:5413–5416.
33. **Wahl, S. M., D. A. Hunt, L. M. Wakefield, N. McCartney-Francis, L. M. Wahl, A. B. Roberts, and M. B. Sporn.** 1987. Transforming growth factor-β (TGF-β) induces monocyte chemotaxis and growth factor production. Proc. Natl. Acad. Sci. USA **84**:5788–5792.
34. **Wilcox, J. N., C. E. Gee, and J. L. Roberts.** 1984. *In situ* cDNA: mRNA hybridization: development of a technique to measure mRNA levels in individual cells. Methods Enzymol. **124**:510–533.