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Developmental Expression of Rat Transforming Growth Factor-α mRNA

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Expression of the gene encoding transforming growth factor-α (TGFα) was examined in developing rat embryos by using a cloned TGFα cDNA as a hybridization probe. Northern blot analysis of RNA isolated from whole fetuses revealed that TGFα mRNA was present at relatively high levels in 8- through 10-day-old embryos and then declined to the low or undetectable level, which is characteristic of adult tissues before birth. The level of TGFα mRNA present during early gestation was similar to that present in retrovirus-transformed cells in culture, suggesting that TGFα expression is not highly localized in the embryo. These observations are consistent with the hypothesis that TGFα plays a role in development, possibly as a fetal growth factor.

Transforming growth factors (TGFs) are potent mitogenic polypeptides produced by a variety of retrovirus-transformed cells and certain tumors (17). In addition, TGFs confer on normal cells in culture some of the phenotypic properties associated with transformation, including loss of contact inhibition and growth in soft agar (3). Two distinct types of TGFs have been identified. TGFα is structurally related to epidermal growth factor (EGF) (6, 11, 12, 15) and competes for binding to the EGF receptor (13). TGFβ, on the other hand, potentiates the growth-stimulating activity of TGFα and demonstrates no affinity for the EGF receptor (1).

Recently, cDNA clones encoding human (4) and rat (9) TGFα's have been isolated; the nucleotide sequence of these cDNAs suggests that human and rat TGFα's are initially synthesized as polypeptides of 160 and 159 amino acids, respectively, and that these larger forms exist as transmembrane proteins. Release of the smaller TGFα from the larger form apparently does not occur through the action of a serine-like protease but, rather, through an unusual proteolytic cleavage between alanine and valine residues at both the amino and carboxy termini of the rat and human molecules.

Using the cloned rat TGFα cDNA as a hybridization probe, we have previously shown that retrovirus-transformed cells express a 4.5-kilobase TGFα mRNA (9). A similar-sized transcript is also detected at considerably lower levels in some adult rat tissues but not in the corresponding untransformed cell line. Here we report that Northern analysis of developing rat embryos indicates that TGFα transcripts are expressed at relatively high levels during early embryogenesis but then decline to low levels before birth. These results are consistent with the hypothesis that TGFα functions as a developmental growth factor.

Pools of rat embryos were collected at various time points from time-mated Sprague-Dawley rats. RNA was isolated by homogenization of embryos or cells in 4 M guanidine thiocyanate and sedimentation through 5.7 M CsCl (2, 5). Poly(A)$^+$ RNA was selected by chromatography on oligo(dT)-cellulose, and samples (5 μg) were electrophoresed through 1% agarose gels containing formaldehyde (10). RNA was transferred to nitrocellulose and probed with the nick-translated TGFα cDNA insert from prTGF0,2 (9). Hybridization was carried out at 45°C in 50% formamide–1× Denhardt reagent–5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–25 mM NaH_2PO_4–herring sperm DNA (200 μg/ml) and washes were performed in 2× SSC–0.1% sodium dodecyl sulfate at 68°C. At each time point, the quality of the poly(A)$^+$ RNA was independently assessed by electrophoresis through 1% agarose-8 M urea gels, followed by staining with ethidium bromide.

Figure 1 shows a Northern analysis of poly(A)$^+$ RNA isolated from pools of whole rat embryos at various stages of development. It is apparent that rat embryos contain, during the early stages of embryogenesis, a TGFα transcript identical in size to the TGFα mRNA contained in retrovirus-transformed rat cells (9; see Fig. 2). The levels of this transcript are highest in 8- through 10-day-old embryos (Fig. 1, lanes A to C) and then decline, beginning at day 11, (lane

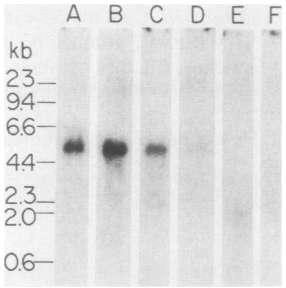

FIG. 1. Northern blot analysis of embryonic rat poly(A)$^+$ RNAs probed with a cloned TGFα cDNA. Poly(A)$^+$ RNAs are from pools of whole 8-day-old (lane A), 9-day-old (lane B), 10-day-old (lane C), 11-day-old (lane D), 13-day-old (lane E), and 18-day-old (lane F) rat embryos. The markers (in kilobases) are *Hin*dIII-digested λDNA.

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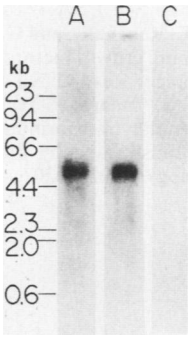

FIG. 2. Northern blot analysis of rat poly(A)$^+$ RNAs probed with the TGFα cDNA clone. Samples are from a pool of whole 9-day-old rat embryos (lane A) and feline sarcoma virus-transformed (lane B) and untransformed (lane C) Fischer rat embryo cells. The markers (in kilobases) are *Hin*dIII-digested λDNA.

D) to undetectable levels by day 13 (lane E) and day 18 (lane F). In other experiments, we have observed that TGFα mRNA sequences can be detected at day 18, but they are consistently present at much lower levels than at the earlier stages of development (data not shown). In addition, we have repeatedly observed that the levels of TGFα mRNA are higher at day 9 than at either day 8 or day 10, suggesting that it represents a peak of TGFα expression; we have not examined embryos before day 8 due to the difficulty in obtaining pure material.

The levels of TGFα transcripts present in 9-day-old embryos (Fig. 2, lane A) are similar to those observed in a clone of feline sarcoma virus-transformed rat fibroblasts (lane B) originally isolated because of its aggressive growth in soft agar and relatively high production of TGFα (19). Lane C contains, for comparison, an equivalent amount of poly(A)$^+$ RNA isolated from the untransformed rat fibroblast cell line. The finding that poly(A)$^+$ RNA from whole rat embryos contains levels of TGFα mRNA similar to those found in a relatively high-producing cell line suggests that TGFα expression in the fetus is not highly localized.

The observation that rat embryos contain relatively high levels of TGFα transcripts suggests that TGFα plays an important role in normal development. Viewed in this light, the abnormal expression of TGFα in the transformed state is analogous to the abnormal expression of developmentally regulated oncogenes (16). Although the appearance of TGFα transcripts in embryos does not necessarily imply that these transcripts are translated to produce TGFα polypeptide, our results are consistent with the earlier observation of Nexo et al. (14) that fetal mice at 11 days of gestation contained material that was capable of binding the EGF receptor but was not recognized in an EGF radioimmunoassay. Subsequently, Twardzik et al. (18) showed that growth factors with some of the characteristic properties of TGFα could be isolated from 12- to 13-day-old mouse embryos. The observation here that rat embryos of a similar gestational age contain TGFα transcripts supports the hypothesis that this growth factor activity derives from the embryo and is not of maternal origin. Finally, it is of interest to note that the growth factor activity isolated from mouse by Twardzik et al. (18) comprised two size classes with apparent molecular weights of 20,000 and 10,000. The relationship of the larger activity to the initial translation product predicted by the TGFα cDNA sequence, as well as the structural form of TGFα expressed in the rat embryos, is presently unclear.

The expression of TGFα in embryos suggests that it might have a normal function as a fetal growth factor, possibly as a developmental analog of EGF. Interestingly Nexo et al. (14) found a significant rise in immunoreactive mouse EGF between days 15 and 17. This is approximately the time when in rat gestation the levels of TGFα mRNA have declined. An alternate explanation is suggested by studies which implicate the action of TGFα in the process of bone resorption (7, 8). Consistent with this model, the expression of TGFα in embryos may be instrumental in the embryonic remodeling which occurs during development.

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