Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 4684–4688, August 1983
Biochemistry

Transforming growth factors produced by retrovirus-transformed rodent fibroblasts and human melanoma cells: Amino acid sequence homology with epidermal growth factor

(serum-free medium/peptide hormone/HPLC purification/radioreceptor assay)

HANS MARQUARDT*, MICHAEL W. HUNKAPILLER†, LEROY E. HOOD†, DANIEL R. TWARDZIK*, JOSEPH E. DE LARCO*, JOHN R. STEPHENSON*, AND GEORGE J. TODARO*

*Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, Maryland 21701; and †Division of Biology, California Institute of Technology, Pasadena, California 91125

Contributed by Leroy E. Hood, May 2, 1983

ABSTRACT **Transforming growth factors (TGFs) were purified from serum-free medium conditioned by retrovirus-transformed Fisher rat embryo fibroblasts, mouse 3T3 cells, and two human melanoma cell lines. The purification of each TGF was monitored in a radioreceptor assay based on receptor crossreactivity with mouse submaxillary gland epidermal growth factor (mEGF) and was achieved by gel permeation chromatography of the acid-soluble TGF-containing activity, followed by reverse-phase high-pressure liquid chromatography with sequential use of acetonitrile and 1-propanol in the presence of aqueous trifluoroacetic acid. The amino-terminal sequences of rat, mouse, and human TGFs were determined. Extensive sequence homology was found among TGF polypeptides from different species and cell types. Alignment of the amino acid sequences of rat TGF, mEGF, and human urogastrone (hEGF) reveals statistically significant sequence homology. The reported results suggest that TGFs that compete for binding to the cellular EGF receptor and EGF may have evolved from a common progenitor.**

Polypeptide growth factors that are able to confer the transformed phenotype on normal cells have been termed transforming growth factors (TGFs) (1). The transformed phenotype is operationally defined (2) by the loss of density-dependent inhibition of cell growth in monolayer culture, overgrowth in monolayer culture, characteristic change in cellular morphology, and acquisition of anchorage-independent growth. TGFs have been classified into two categories on the basis of their biological and physical properties (3). Each member of the type I TGFs (TGFα) competes with epidermal growth factor (EGF) for binding to the cellular EGF receptor. In contrast, type II TGFs (TGFβ) display no measurable binding to EGF receptors but may potentiate the growth-stimulating activity of type I TGFs in semisolid medium (4). Transformed cells cultured in serum-free medium produce and release growth factors with the ability to confer the transformed phenotype on normal fibroblasts (2) and epithelial cells (5) *in vitro*. Polypeptides with the properties of a type I TGF have been isolated and partially purified from the conditioned medium of Moloney murine sarcoma virus-transformed mouse 3T3 cells (6), from retrovirus-transformed rat fibroblasts (7), and from certain human tumor cells (8). A low molecular weight human melanoma-derived type I TGF (hTGF) has been purified to apparent homogeneity (9). Both type I hTGF and mouse submaxillary gland EGF (mEGF) are qualitatively and quantitatively nearly indistinguishable in competing for binding to (9) and for inducing tyrosine phosphorylation of (10) the EGF receptor and in stimulating the phosphorylation, by A431 human carcinoma and mouse 3T3 cell membranes, of a synthetic tyrosine-containing peptide (11).

We describe in this paper the large-scale purification of low molecular weight rat and mouse type I TGFs (rTGF and mTGF) and of hTGF that are produced by Snyder–Theilen feline sarcoma virus-transformed Fisher rat embryo fibroblasts, Moloney murine sarcoma virus-transformed mouse 3T3 cells, and two human melanoma cell lines, respectively. The purification of each TGF was monitored in a radioreceptor assay based on receptor crossreactivity with mEGF. The amino-terminal sequences of the isolated polypeptides are reported. The data clearly define the structural relationship among these functionally indistinguishable type I TGF polypeptides and establish unequivocally a structural relationship to EGF.

MATERIALS AND METHODS

Source of TGF. rTGF, mTGF, and hTGF were purified from the serum-free medium conditioned respectively by Fisher rat embryo fibroblasts FRE Cl10, a subclone of FRE 3A (12), nonproductively transformed by Snyder–Theilen feline sarcoma virus (13); by a Moloney murine sarcoma virus-transformed mouse 3T3 cell line, 3B11-IC (14); and by two human metastatic melanoma lines, A2058 (8) and A375 (15). Cells were grown in 2-liter plastic roller bottles containing Dulbecco's modified Eagle's medium supplemented with 10% calf serum and subsequently maintained in serum-free Waymouth's medium as described (2). Serum-free conditioned medium was collected every 24 hr for a 3-day period and clarified by continuous flow centrifugation. The supernatant was concentrated (16), and this concentrate of conditioned medium was the starting material for the purification of TGFs.

Purification of TGF. rTGF and mTGF were prepared essentially as described (9) for the purification of the melanoma-derived hTGF. The retentate after ultrafiltration of conditioned medium was dialyzed against 0.1 M acetic acid, and the supernatant, after centrifugation, was concentrated by lyophilization and reconstituted in 1 M acetic acid for subsequent gel permeation chromatography on a column (2.5 × 85 cm) of Bio-Gel P-10 (200–400 mesh, Bio-Rad Laboratories). The column was equilibrated with 1 M acetic acid. Fractions comprising the major EGF-competing activity and having an apparent molecular weight of approximately 7,000 were pooled and lyophilized.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: TGF, transforming growth factor; rTGF, mTGF, and hTGF, low molecular weight rat, mouse, and human transforming growth factors [the term mTGF will refer in this communication to the low molecular weight sarcoma growth factor (6)]; EGF, epidermal growth factor; mEGF, mouse submaxillary gland EGF; hEGF, urogastrone.

The final purification of rTGF, mTGF, and hTGF was achieved by reverse-phase HPLC. The chromatography system has been described (17). The separations were performed on a μBondapak C_{18} column (10-μm particle size, 0.39 × 30 cm, Waters Associates). The mobile phase was 0.05% trifluoroacetic acid, and the mobile-phase modifier was acetonitrile containing 0.045% trifluoroacetic acid. The concentration of acetonitrile was increased linearly (0.083%/min) during 2 hr at a flow rate of 1 ml/min at 40°C for elution of peptides. TGF-containing pools were lyophilized and reconstituted in 0.05% trifluoroacetic acid and were rechromatographed on the same column, with 1-propanol containing 0.035% trifluoroacetic acid as the mobile-phase modifier. The 1-propanol concentration was increased linearly (0.05%/min) during 2 hr at a flow rate of 1 ml/min at 40°C. Pools of fractions comprising the major EGF-competing activity were lyophilized.

Assay for TGF. TGF was quantitated in a radioreceptor assay based on receptor crossreactivity with mEGF. Purified mEGF (18) was labeled with $Na^{125}I$ (^{125}I-EGF) by a modification of the chloramine-T method (19) as described (6). The ^{125}I-EGF binding assay was performed on Formalin-fixed A431 (15) human carcinoma cells (8×10^3) in Micro Test II plates (Falcon) (9). The concentration of TGF was expressed in nanogram equivalents of mEGF per ml and was based on the amount of TGF required to produce equal inhibition of ^{125}I-EGF binding to A431 cells as a known amount of unlabeled mEGF.

Amino Acid Sequence Determination of TGF. For amino-terminal sequence analysis, rTGF (3 μg) was reduced with dithiothreitol (20 mM) in 100 μl of 0.4 M Tris·HCl/6 M guanidine·HCl/0.1% Na_2EDTA, pH 8.5, for 2 hr at 50°C and subsequently S-carboxamidomethylated with iodoacetamide (45 mM) for 30 min at 22°C. The S-carboxamidomethylated rTGF was desalted on a μBondapak C_{18} column. Peptide was eluted with a gradient of aqueous acetonitrile containing 0.045% trifluoroacetic acid. The concentration of acetonitrile was increased linearly (1%/min) during 1 hr at a flow rate of 1 ml/min at 40°C.

Automated sequence analyses (20) of S-carboxamidomethylated rTGF and unmodified mTGF and hTGF were performed with a gas/liquid solid-phase microsequenator (21). Sequenator fractions were analyzed by reverse-phase HPLC (22).

Statistical Analyses for Relatedness of the TGF Amino Acid Sequence to Other Proteins. The partial amino acid sequence of rTGF was compared with each known sequence stored in a protein sequence data base (23) using the SEARCH program (24). Homologies between the rTGF sequence and those of mEGF (25) and human urogastrone (hEGF) (26) were established by using the program ALIGN (27). The program ALIGN determines a best alignment of two protein sequences (including gaps) by computing the maximum match score using the mutation data matrix (250 PAMs) (28) with a matrix bias parameter and a penalty parameter each set to 6. This score is then compared with the highest possible scores obtained by aligning pairs of 100 random formulations having the same amino acid composition as the two real sequences. The scores of the randomized sequences form a normal distribution for which the mean and standard deviation are calculated. The probability that the score for the real sequences is derived from this normal distribution is then obtained.

RESULTS

Purification of TGF. Purified preparations of a low molecular weight rTGF, mTGF, and hTGF were obtained from the conditioned medium of retrovirus-transformed rat and mouse fibroblasts and two human melanoma cell lines, respectively. The purification was achieved by gel permeation chromatography of the acid-soluble EGF-competing activity on Bio-Gel P-10 in 1 M acetic acid, followed by reverse-phase HPLC on μBondapak C_{18} support with sequential use of a linear gradient of aqueous acetonitrile and 1-propanol containing trifluoroacetic acid. Typical elution patterns of the final purification step of rTGF, mTGF, and hTGF are shown in Fig. 1. EGF-competing activity copurified with a distinct absorbance peak, indicated with a bar, and was effectively separated from contaminating UV-absorbing material. The major protein peak in rTGF,

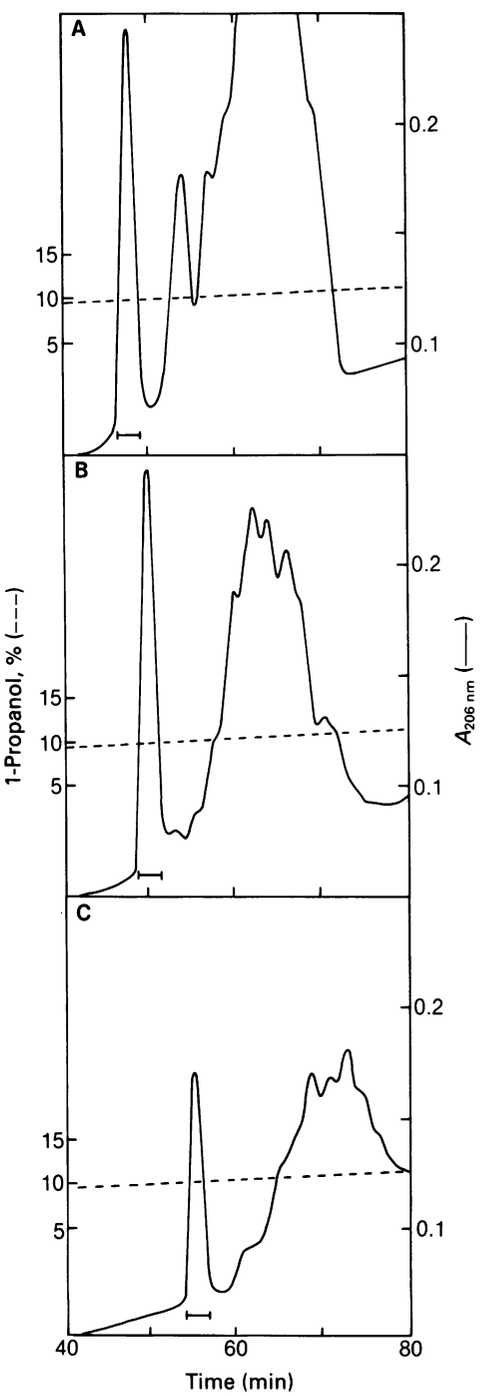

FIG. 1. Final purification of rTGF (*A*), mTGF (*B*), and hTGF (*C*) by reverse-phase HPLC on μBondapak C_{18} support. The elution of peptides was achieved with a 10-min linear gradient of 0–8% 1-propanol, followed by a 2-hr linear gradient of 8–14% 1-propanol containing 0.035% trifluoroacetic acid. The 1-propanol concentration was increased (0.05%/min) during 2 hr at a flow rate of 1 ml/min at 40°C. UV-absorbing material was detected at 206 nm. The horizontal bar indicates pooled TGF.

mTGF, and hTGF preparations was eluted from a μBondapak C_{18} column under standard conditions between 48 and 55 min.

Gel permeation chromatography on Bio-Gel P-10 provided a separation of the low molecular weight TGFs from larger molecular weight TGFs and reduced the load of protein applied to a μBondapak C_{18} column in the following purification step. The low molecular weight TGFs represented 45–80% of the initial total EGF-competing activity. Reverse-phase HPLC of TGFs on μBondapak C_{18} support in the following two purification steps was very efficient, each step in a typical preparation giving a recovery range of 80–100%. The final recovery of the low molecular weight TGFs was approximately 70%, based on the maximal total EGF-competing activity detected during the course of the purification. The average yield per liter of conditioned medium of purified rTGF was 90 ng, of mTGF was 50 ng, and of hTGF was 10 ng. This calculation is based on the specific activity determined for isolated TGFs and on the assumption that the EGF-competing activity measured in the radioreceptor assay reflects levels of total large and low molecular weight TGFs only. No immunoreactive mEGF was detected in conditioned medium (2).

Purity of TGF. The purity of rTGF, mTGF, and hTGF suggested by the chromatographic elution profiles was assessed in the EGF radioreceptor assay and by amino acid sequence analysis. rTGF, mTGF, and hTGF competed with ^{125}I-EGF for the EGF receptor sites on A431 human carcinoma cells and were qualitatively and quantitatively nearly indistinguishable from mEGF. Hence, the final TGF preparations were believed to be highly purified and close to homogeneity. A single amino-terminal sequence was determined by automated Edman degradation for rTGF, mTGF, and hTGF. Any unblocked minor peptide sequence present at >5% could have been detected by the methods used. The homogeneity of hTGF was confirmed in addition by analytical $NaDodSO_4$/polyacrylamide gel electrophoresis. The purified preparation gave one major polypeptide band (9).

Amino-Terminal Sequence of TGF. Automated Edman degradation of rTGF was performed with 300 pmol (based on the initial yield of identified phenylthiohydantoin-amino acids) of the S-carboxamidomethylated derivative. Unambiguous identification of phenylthiohydantoin derivatives of amino acids was possible up to residue 43, except at positions 26, 30, 36, 40, and 42, where no amino acid could be assigned. The residues at positions 28 and 37 were tentatively assigned. The amino-terminal amino acid sequence of rTGF is given in Fig. 2.

The amino-terminal sequence of mTGF was determined with 550 pmol of unmodified mTGF. Amino acid residues at positions 8, 16, 21, 30, 32, 34, and 36 were not identified in this experiment. The residues at positions 26, 28, and 37 were tentatively assigned. The partial amino-terminal amino acid sequence of mTGF is shown in Fig. 2.

The sequence of melanoma-derived hTGF was determined twice. Edman degradation of unmodified hTGF, isolated from A375-conditioned medium, was performed with 120 pmol for 34 cycles. The results are shown in Fig. 2. Amino acid residues at positions 8, 16, 21, 22, 23, 26, 30, 32, and 34 were not identified. Residues at positions 20, 24, 28, and 33 were tentatively assigned. The amino-terminal sequence of unmodified hTGF, isolated from A2058-conditioned medium, was determined with 75 pmol. Identified phenylthiohydantoin derivatives of amino acids up to residue 19 were identical with those established in the partial sequence of A375-derived hTGF.

Amino-Terminal Sequence Homology of rTGF with mEGF and hEGF. The sequence of rTGF was entered into the protein data base of the *Atlas of Protein Sequence and Structure* in January 1983, and the SEARCH program was used to compare residues 1–25 and 19–43 against the current protein data base, containing 2,145 protein sequences. Statistically significant sequence homology was found only between mEGF, hEGF, and rTGF. The possible structural relatedness between these polypeptides was statistically analyzed by using the program ALIGN. The alignment of the amino-terminal sequence of rTGF with sequences of mEGF and hEGF is shown in Fig. 3. The alignment scores, *A*, from intercomparisons of rTGF, mEGF, and hEGF are shown in Table 1. The probabilities of rTGF being so similar by chance to mEGF and hEGF are $<10^{-11}$.

DISCUSSION

Type I TGFs were isolated from serum-free medium conditioned by retrovirus-transformed rat and mouse fibroblasts and human melanoma cells. The purification of TGFs was monitored in a radioreceptor assay developed for mEGF and was achieved by gel permeation chromatography and reverse-phase HPLC with sequential use of mobile-phase modifiers of different solvent polarity in the presence of the same hydrophobic counterion. TGFs could be directly identified in the last purification step by determining the characteristic elution positions of rTGF, mTGF, and hTGF when chromatographed on μBondapak C_{18} support (Fig. 1) and quantitated by integrating peak areas. The recovery of isolated TGFs from conditioned medium was about 70%.

Comparison of the amino-terminal sequences of rTGF, mTGF, and hTGF revealed a high degree of sequence homology. When the sequences were aligned, as shown in Fig. 2, all of the identified residues in rTGF and mTGF were identical. A comparison of the amino-terminal sequence of rTGF with the amino-terminal sequence of hTGF indicated that hTGF and rTGF differed from each other by at least three amino acid substitutions. The sequence of hTGF differed from that of rTGF by substitution of aspartic acid for lysine in position 7, a conservative substitution of phenylalanine for tyrosine in position 15, and a substitution in position 23, although not positively identified; 23 of 26 amino acid positions, or 88%, are identical.

```
          1     5     10    15    20    25    30    35    40
rTGF  V V S H F N K C P D S H T Q Y C F H G T C R F L V X E(E)K X A C V C H X(G)Y V X V X C

mTGF  V V S H F N K X P D S H T Q Y X F H G T X R F L V(Q)E(E)K X A X V X H X(G)Y V X

hTGF  V V S H F N D X P D S H T Q F X F H G(T)X X X(L)V X E(E)K X A X(V)X
```

FIG. 2. Alignment of amino-terminal sequences of rTGF, mTGF, and hTGF. Amino acid residues are given in single letter code (29). Residues in the amino acid sequence of hTGF that differ from those in sequences of rTGF and mTGF are underlined. X, Unidentified residue.

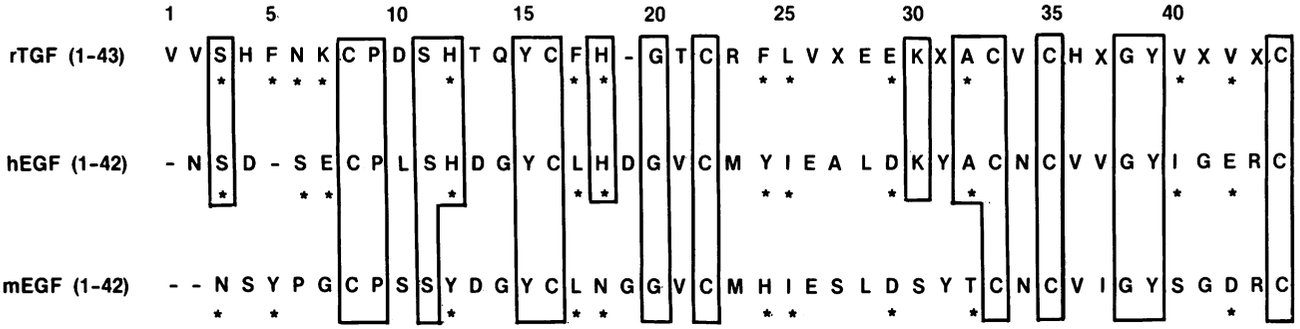

FIG. 3. Alignment of amino acid sequences of rTGF, mEGF (25), and hEGF (26). Numbers in parentheses denote actual residues compared. Amino acid residues are given in single letter code (29). Hyphens indicate gaps introduced to bring homologous regions of the structures into alignment. Boxes indicate regions or residues of identity in rTGF, mEGF, and hEGF. Residues differing in the genetic code by only one base are underscored by an asterisk.

Edman degradation of unmodified mTGF and hTGF did not give identifiable phenylthiohydantoin-amino acids for residues 8, 16, 21, 32, and 34. The demonstrated sequence similarity of rTGF with mTGF and hTGF can be taken as presumptive evidence that half-cystine/cysteine residues, which are nondetectable on analysis of native proteins, occupied these positions in the amino acid sequences of mTGF and hTGF.

The data presented here indicate that rTGF, mTGF, and hTGF may differ from each other by only a few amino acid substitutions. These results establish that TGFs produced by retrovirus-transformed rat and mouse fibroblasts and two human melanoma cell lines are highly conserved structurally and functionally among different species and cell types. The similarities in the amino-terminal sequences of rTGF and mTGF produced by cells transformed by two different retroviruses containing genetically distinct acquired cellular sequences favor the conclusions that TGFs represent cellular rather than viral gene products and that TGF production may be relatively common in transformed cells.

An interesting feature of the amino-terminal sequence of rTGF is the structural relationship to hEGF and mEGF. A computer-assisted alignment (Fig. 3) of the first 43 amino acid residues of rTGF with those of hEGF resulted in 17 common amino acid residues out of 36 possible comparisons, yielding an alignment score of 7.85 SD. When the sequences of rTGF and mEGF were compared, an alignment score of 7.24 SD was obtained, which is well above the range of random chance. Moreover, there is considerable conservation of third-base-coding assignments. Between the amino-terminal sequences of rTGF and hEGF, 8 of 36 residues differed in the genetic code by only one base; 10 of 36 third-base identities are found between rTGF and mEGF. Alignment of the amino-terminal sequences of rTGF with hEGF and mEGF required a single deletion between residues 18 and 20 to bring all six cysteines into register, thus maintaining the overall disulfide bond configuration.

The observed homology between type I TGFs and EGFs emphasizes evolutionary relationship and suggests that type I TGFs and EGFs may have evolved through a process of gene duplication from a common ancestral molecule. The divergence between type I TGFs and EGFs may have occurred before the appearance of the vertebrates, provided that the rate of evolutionary change was similar in the two polypeptides. The degree of homology detected between type I TGFs and mEGF is not sufficient to cause immunologic crossreactivity, thus confirming earlier observations (2). Likewise, type I mTGF does not compete with mEGF for binding to the specific and saturable EGF-binding protein (30) (unpublished data), which functions in the enzymatic processing of active mEGF from an inactive precursor (31). Therefore, type I TGFs and mEGF appear to share structurally a common cellular receptor binding region only. The large sequence differences observed between type I TGFs and EGFs suggest that during peptide hormone evolution, type I TGFs acquired additional structural properties. Evidence was presented (32) that type I TGFs can bind specifically to a different set of receptors on normal rat kidney cells than does mEGF. The interaction of type I TGFs with this receptor is thought to induce the transformed phenotype expressed by these untransformed cells.

A physiological role of type I mTGF and rTGF in neoplastic transformation was suggested largely by correlating their production with the expression of the transformed phenotype in rodent cells by use of well-characterized viral mutants (33, 34) but has not yet been shown for type I hTGF produced by human tumor cells. The expression of type I TGFs in neoplastic tissues (35) and the ectopic production by transformed cells may be a benefit to the cell and could then play a vital part in the growth and development of tumors.

Table 1. Alignment scores for comparison of rTGF, mEGF, and hEGF

	Alignment score, SD units	
	rTGF (1–43)	mEGF (1–42)
rTGF (1–43)	—	7.24
hEGF (1–42)	7.85	13.86

Alignment scores were obtained by using the program ALIGN. Numbers in parentheses denote actual residues compared. The mEGF sequence was from ref. 25, the hEGF sequence was from ref. 26.

We are grateful to Dr. Winona C. Barker for the statistical analyses, to John P. Bordonaro and Jane Ranchalis for able technical assistance, to Dolores Winterstein and E. Siobhan McCormack for preparing conditioned medium, and to Virginia Frye for typing this manuscript.

1. Sporn, M. B., Newton, D. L., Roberts, A. B., De Larco, J. E. & Todaro, G. J. (1981) in *Molecular Actions and Targets for Cancer Chemotherapeutic Agents*, eds. Sartorelli, A. C., Bertino, J. R. & Lazo, J. S. (Academic, New York), pp. 541–554.
2. De Larco, J. E. & Todaro, G. J. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4001–4005.
3. Roberts, A. B., Anzano, M. A., Lamb, L. C., Smith, J. M., Frolik, C. A., Marquardt, H., Todaro, G. J. & Sporn, M. B. (1982) *Nature (London)* **295**, 417–419.
4. Anzano, M. A., Roberts, A. B., Meyers, C. A., Komoriya, A., Lamb, L. C., Smith, J. M. & Sporn, M. B. (1982) *Cancer Res.* **42**, 4776–4778.
5. Keski-Oja, J., De Larco, J. E., Rapp, U. R. & Todaro, G. J. (1980) *J. Cell Physiol.* **104**, 41–46.

6. De Larco, J. E., Reynolds, R., Carlberg, K., Engle, C. & Todaro, G. J. (1980) *J. Biol. Chem.* **255,** 3685–3690.
7. Ozanne, B., Fulton, J. & Kaplan, P. L. (1980) *J. Cell Physiol.* **105,** 163–180.
8. Todaro, G. J., Fryling, C. & De Larco, J. E. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5258–5262.
9. Marquardt, H. & Todaro, G. J. (1982) *J. Biol. Chem.* **257,** 5220–5225.
10. Reynolds, F. H., Jr., Todaro, G. J., Fryling, C. & Stephenson, J. R. (1981) *Nature (London)* **292,** 259–262.
11. Pike, L. J., Marquardt, H., Todaro, G. J., Gallis, B., Casnellie, J. E., Bornstein, P. & Krebs, E. G. (1983) *J. Biol. Chem.* **257,** 14628–14631.
12. Sacks, T. L., Hershey, E. J. & Stephenson, J. R. (1979) *Virology* **97,** 231–240.
13. Snyder, S. P. & Theilen, G. H. (1969) *Nature (London)* **221,** 1074–1075.
14. Bassin, R. M., Tuttle, N. & Fischinger, P. J. (1970) *Int. J. Cancer* **6,** 95–107.
15. Giard, D. J., Aaronson, S. A., Todaro, G. J., Arnstein, P., Kersey, J. H., Dosik, H. & Parks, W. P. (1973) *J. Natl. Cancer Inst.* **51,** 1417–1423.
16. Marquardt, H., Wilson, G. L. & Todaro, G. J. (1980) *J. Biol. Chem.* **255,** 9177–9181.
17. Marquardt, H., Todaro, G. J., Henderson, L. E. & Oroszlan, S. (1981) *J. Biol. Chem.* **256,** 6859–6865.
18. Savage, C. R., Jr., & Cohen, S. (1972) *J. Biol. Chem.* **247,** 7609–7611.
19. Hunter, W. M. & Greenwood, F. C. (1962) *Nature (London)* **194,** 495–496.
20. Edman, P. & Begg, G. (1967) *Eur. J. Biochem.* **1,** 80–91.
21. Hewick, R. M., Hunkapiller, M. W., Hood, L. E. & Dreyer, W. J. (1981) *J. Biol. Chem.* **256,** 7990–7997.
22. Hunkapiller, M. W. & Hood, L. E. (1983) *Science* **219,** 650–659.
23. Dayhoff, M. O., Hunt, L. T., Barker, W. C., Orcutt, B. C., Yeh, L.-S., Chen, H. R., George, D. G., Blomquist, M. C. & Johnson, G. C. (1983) *Protein Sequence Database* (National Biomedical Research Foundation, Washington, DC).
24. Dayhoff, M. O. (1979) in *Atlas of Protein Sequence and Structure*, ed. Dayhoff, M. O. (National Biomedical Research Foundation, Washington, DC), Vol. 5, Suppl. 3, pp. 1–8.
25. Savage, C. R., Jr., Inagami, T. & Cohen, S. (1972) *J. Biol. Chem.* **247,** 7612–7621.
26. Gregory, H. (1975) *Nature (London)* **257,** 325–327.
27. Barker, W. C. & Dayhoff, M. O. (1972) in *Atlas of Protein Sequence and Structure*, ed. Dayhoff, M. O. (National Biomedical Research Foundation, Washington, DC), Vol. 5, Suppl. 3, pp. 101–110.
28. Schwartz, R. M. & Dayhoff, M. O. (1979) in *Atlas of Protein Sequence and Structure*, ed. Dayhoff, M. O. (National Biomedical Research Foundation, Washington, DC), Vol. 5, Suppl. 3, pp. 353–358.
29. IUPAC-IUB Commission on Biochemical Nomenclature (1968) *J. Biol. Chem.* **243,** 3557–3559.
30. Taylor, J. M., Mitchell, W. M. & Cohen, S. (1974) *J. Biol. Chem.* **249,** 2188–2194.
31. Frey, P., Forand, R., Maciac, T. & Shooter, E. M. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 6294–6298.
32. Massague, J., Czech, M. P., Iwata, K., De Larco, J. E. & Todaro, G. J. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6822–6826.
33. De Larco, J. E., Preston, Y. A. & Todaro, G. J. (1981) *J. Cell Physiol.* **109,** 143–152.
34. Twardzik, D. R., Todaro, G. J., Marquardt, H., Reynolds, F. H., Jr., & Stephenson, J. R. (1982) *Science* **216,** 894–897.
35. Roberts, A. B., Lamb, L. C., Newton, D. L., Sporn, M. B., De Larco, J. E. & Todaro, G. J. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3494–3498.