

## Isolation of a cDNA Corresponding to a Developmentally Regulated Transcript in Rat Intestine

J. FILMUS, J. G. CHURCH, AND R. N. BUICK\*

*Ontario Cancer Institute, and Department of Medical Biophysics, University of Toronto,  
500 Sherbourne Street, Toronto, Ontario M4X 1K9, Canada*

Received 12 May 1988/Accepted 28 June 1988

**We report the isolation of a cDNA clone corresponding to a transcript that is accumulated differentially in rat intestine during development. Clone OCI-5 was selected from the rat intestinal cell line IEC-18, which represents primitive intestinal epithelial crypt cells. Expression was high in rat fetal intestine between 15 and 19 days of development and thereafter was progressively down regulated, becoming undetectable after weaning. Clone OCI-5 detected homologous sequences in human and murine cells. In particular, a high level of expression was detected in CaCo-2, a human colon carcinoma cell line, which is known to express molecules characteristic of fetal small intestinal cells. Expression of a homologous gene was also detected in F9 murine teratocarcinoma cells when they were induced to differentiate into parietal or visceral endodermlike cells. When IEC-18 cells were transformed by activated *H-ras* or *v-src* genes, expression of clone OCI-5 was suppressed; the degree of down-regulation correlated with the extent of morphological change induced in the transformed IEC-18 cells. The sequence of clone OCI-5 showed an open reading frame that was capable of encoding a protein of 597 amino acids, but no strong homology was found with any of the proteins registered in the protein sequence data base.**

The normal epithelial mucosa of the small intestine is organized as a single layer of cells lining the lumen of the gut. The tissue function of transport and digestion is performed by the absorptive cells, which are arranged on villi that protrude into the gut lumen. The half-life of these cells is short; and cell number is constantly maintained by proliferation, differentiation, and migration of cells from the crypts, which are pockets of the epithelium arranged below the surface of the gut. Results of studies by Cheng and Leblond (6) in the mouse have established the concept that cell renewal is achieved through an amplifying cell lineage originating in crypt stem cells which have the potential for differentiation into the four main types of differentiated cells: absorptive, goblet, endocrine, and Paneth cells. Based on kinetic evidence, the stem cells are located close to the base of each crypt and likely number less than 16 per crypt, a very small proportion of the total number of cells in the intestinal epithelium (26).

The mature absorptive cells, which are the predominant cell population on the surface of the villi, acquire a distinctive brush border membrane and surface membrane enzymes which have been studied extensively at the cellular and molecular levels. However, the crypt cells, including those assumed to represent the stem cells, remain relatively poorly characterized. Quaroni (27, 29) has prepared several monoclonal antibodies against membranes of intestinal cells that react specifically against crypt cells in both rat and human adult small intestines, but the antigens have not yet been characterized.

The morphogenesis of intestinal mucosa is a late event in gestation (17). At day 15 of development the small and large intestines in fetal rats consist of an undifferentiated stratified epithelium within a mesenchymal layer. Thereafter, primitive villi are formed and progressively covered by a single layer of columnar epithelial cells. Crypt cells appear at about

the time of birth, and complete maturation is reached only after weaning. DNA synthesis and cell proliferation occur in all intestinal cells at earlier stages of development, and the restriction of the proliferative zone to the base of the villi and the crypts is first detected 1 day before birth (11). In several studies it has been suggested that adult crypt cells and fetal intestinal cells are not only ultrastructurally but also phenotypically similar (27, 28, 30, 38).

As a first approach to the generation of probes useful to study the molecules that are involved in proliferation and differentiation of crypt stem cells, we decided to isolate cDNA sequences that are expressed in fetal intestinal endoderm and that are down regulated during development. Since it is very difficult to obtain a pure population of fetal epithelial cells from rat intestine, we used a rat intestinal cell line (IEC-18) as a source of mRNA for the cDNA library. IEC-18 is one of a series of rat intestinal cell lines which have been established by Quaroni and Isselbacher (31). Results of morphological and immunological studies have indicated that these cells represent undifferentiated crypt epithelial cells (31). Moreover, one of these cell lines, IEC-17, was shown to have the potential for intestinal-specific differentiation (including differentiation into absorptive, goblet, endocrine, and Paneth cells) when transplanted in conjunction with fetal gut mesenchyme into the kidney capsule of adult rats or into nude mice (13). In addition, an enterocytic type of differentiation can be induced in IEC-6 cells by transforming growth factor  $\beta$  (14).

To eliminate mRNA sequences that could be characteristic of epithelial cells in general or of actively proliferating cells in tissue culture, we screened the IEC cDNA library by a subtractive hybridization technique that was designed to detect sequences that are specifically expressed in IEC-18 cells compared with those that are expressed in a normal rat kidney epithelial cell line (NRK-52E). Using this technique, we were able to isolate a cDNA corresponding to an mRNA which is developmentally regulated in the rat intestine.

\* Corresponding author.

## MATERIALS AND METHODS

**Cell culture.** The following cell lines were used in these experiments: IEC-18 (31) and IEC-14 (32), derived from normal rat small intestine; NRK-52E, derived from normal rat kidney; F9, derived from a mouse teratocarcinoma (35); Rat-2, derived from normal rat fibroblasts (36); CaCo-2, HT-29, and DLD-1, derived from human colon carcinomas; MDA-468, derived from a human breast adenocarcinoma (22); SKMG-3, derived from a human glioblastoma (23); HOC-8, derived from a human ovarian adenocarcinoma (9); and 427N, derived from normal human fibroblasts. NRK-52E, CaCo-2, HT-29, and DLD-1 were obtained from the American Type Culture Collection (Rockville, Md.).

The induction of differentiation of F9 teratocarcinoma cells into visceral and parietal endodermlike cells was performed as described previously (12) by using retinoic acid alone or dibutyl cyclic AMP-retinoic acid, respectively.

**Differential screening.** Double-stranded cDNA was synthesized from poly(A)<sup>+</sup> RNA that was obtained from IEC-18 cells as described previously (21). The cDNA was ligated to *Eco*RI polylinkers and, after *Eco*RI digestion, was cloned into the unique *Eco*RI site of  $\lambda$ gt10 DNA. The recombinant phage library containing 20,000 plaques was dotted onto duplicate nitrocellulose filters (1,000 plaques per filter). For preparations of enriched probes (10), <sup>32</sup>P-labeled single-stranded cDNA that was reverse transcribed from 3  $\mu$ g of IEC-18 poly(A)<sup>+</sup> RNA was annealed for 40 h at 68°C to 25  $\mu$ g of NRK-52E poly(A)<sup>+</sup> RNA in a volume of 12  $\mu$ l, containing 0.5 M sodium phosphate buffer (pH 7.0), 1 mM EDTA, and 0.1% sodium dodecyl sulfate (SDS), in a sealed siliconized glass capillary. This mixture was then diluted into 1 ml of 0.12 M sodium phosphate buffer containing 0.1% SDS and passed over a column of 0.2 g of hydroxyapatite to separate the single-stranded cDNA from the double-stranded cDNA. The eluate containing the single-stranded cDNA was used to screen the 20,000 recombinant phages on nitrocellulose filters. Hybridization was carried out at 65°C in 6 $\times$  SSC (1 $\times$  SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 1 $\times$  Denhardt solution, and 200  $\mu$ g of denatured salmon sperm DNA per ml. The filters were washed at 65°C in 0.1 $\times$  SSC. A duplicate set of nitrocellulose filters was hybridized with a <sup>32</sup>P-labeled single-stranded cDNA prepared from NRK-52E mRNA by using the same conditions as were used for the IEC-18-subtracted probe.

**RNA isolation and blotting.** Total RNA was isolated from cultured cells by guanidine isothiocyanate solubilization and centrifugation over a CsCl cushion. Poly(A)<sup>+</sup> RNA was purified by passage over oligo(dT)-cellulose. The RNA was denatured with glyoxal and dimethyl sulfoxide, and electrophoresis was performed in a 1.1% agarose gel. To verify that equal amounts of RNA were loaded into each lane, the gels were stained with ethidium bromide. Human 28S and 18S rRNAs were used as size markers. The RNA was then transferred to a Zetabind filter and hybridized under high-stringency conditions with clone OCI-5 that was nick translated with <sup>32</sup>P. When the RNA was to be isolated from intestines, the tissue was frozen in liquid nitrogen and pulverized before it was dissolved in guanidine isothiocyanate.

**DNA isolation and blotting.** Genomic DNA was isolated by SDS-proteinase K lysis, organic extraction, and NaCl-ethanol precipitation. DNA was digested with *Hind*III, electrophoresed in a 0.8% agarose gel, and transferred to a Zetabind membrane. Clone OCI-5 was nick translated with <sup>32</sup>P, and hybridization was performed at 42°C in 5 $\times$  SSC, 1 $\times$  Den-

hardt solution, 100  $\mu$ g of denatured salmon sperm DNA per ml, 0.1% SDS, 20 mM sodium phosphate, and 30% formamide.

**DNA sequencing.** *Eco*RI, *Pst*I, *Hind*III, and *Sst*I fragments of clone OCI-5 were purified from an agarose gel and cloned into the bacteriophage M13 mp18 vector (19). Single-stranded DNA templates were prepared as described previously (33) and sequenced by the dideoxynucleotide chain termination method (34). The sequence analysis was completed by the use of seven specific oligonucleotide primers, as indicated by asterisks in Fig. 7A.

**v-src infection of IEC-18 cells.** IEC-18 cells were seeded overnight at a density of 10<sup>5</sup> cells per dish in growth medium. The following day different dilutions of SR1, a retrovirus containing the v-src gene and a gene coding for resistance to G-418 (3), were added in the presence of 8  $\mu$ g of Polybrene per ml of medium. Cells were incubated overnight at 37°C. The following day the medium was replaced by regular medium. After 48 h, 400  $\mu$ g of G-418 was added per ml. Two weeks later transformed foci were cloned and expanded in the presence of G-418.

## RESULTS

**Preparation and differential screening of a cDNA library from IEC-18 cells.** A  $\lambda$ gt10 cDNA library was prepared by using mRNA extracted from exponentially growing IEC-18 cells. A total of 2  $\times$  10<sup>4</sup> independent recombinant plaques were plated at a low density and transferred onto duplicate nitrocellulose filters. <sup>32</sup>P-labeled single-stranded cDNA was prepared from IEC-18 mRNA and submitted to subtractive hybridization against mRNA extracted from NRK-52E. Eighty percent of the IEC-18 cDNA hybridized with the NRK-52E mRNA. The remaining 20% was used to screen one set of the nitrocellulose filters blotted with the IEC-18 cDNA library. The other set of nitrocellulose filters was screened with single-stranded cDNA that was prepared from NRK-52E mRNA. Plaques that were positive when probed with the IEC-18-subtracted cDNA and negative with NRK-52E cDNA were selected for a secondary screening. Five of these plaques showed similar results in the secondary screening and were selected for further studies.

**Characterization of cDNA clones.** DNA was prepared from the five selected plaques, and the cloned inserts were isolated. All of them had the same size (2.25 kilobases [kb]), suggesting that they contained the same sequence. The five inserts were nick translated with <sup>32</sup>P and hybridized to a Northern blot containing mRNA extracted from IEC-18, IEC-14, NRK-52E, 15-day-old fetal rat intestine, adult rat intestine, and a normal rat fibroblast cell line (Rat-2). The same pattern was obtained with the five inserts (a representative pattern is shown in Fig. 1). Enzymatic restriction analysis confirmed that the five clones contained the same sequence. A single 2.6-kb band was detected in IEC-18 cells but was absent from all the other cell lines. The fetal rat intestine also showed a prominent 2.6-kb band, whereas the adult intestine was negative for this band. This last observation indicates that the expression of this 2.6-kb message is developmentally regulated. The insert from one  $\lambda$ gt10 clone was subsequently cloned into the *Eco*RI site of plasmid PUC13 and designated OCI-5.

**Expression studies of clone OCI-5.** To define the temporal relationship between OCI-5 expression and intestinal development, total RNA was extracted from the intestines of embryos and rats of different ages from 15 days of gestation to 24 days postparturition, and a Northern blot was per-

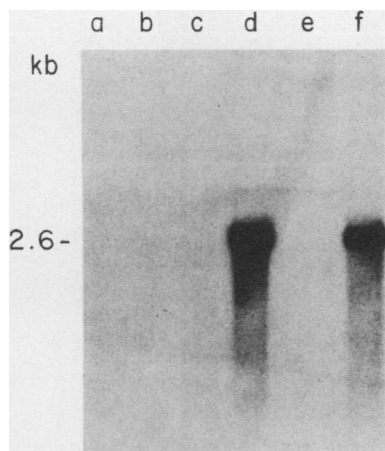


FIG. 1. Northern blot analysis of clone OCI-5. mRNA (3  $\mu$ g) was hybridized with nick-translated OCI-5. Lanes: a, NRK-52E; b, RAT-2; c, adult rat intestine; d, 15-day-old fetal rat intestine; e, IEC-14; f, IEC-18.

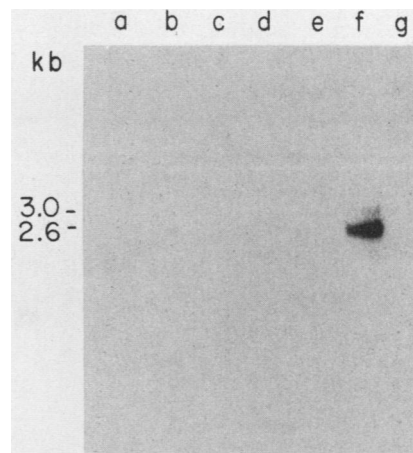


FIG. 3. Northern blot analysis of clone OCI-5 in human cell lines. mRNA (3  $\mu$ g) was hybridized with nick-translated OCI-5. Lanes: a, MDA-468; b, SKMG-3; c, HOC-8; d, 427-N; e, DLD-1; f, CaCo-2; g, HT-29.

formed. Figure 2 shows that the expression of clone OCI-5 decreased gradually from day 20 of gestation and finally became undetectable after weaning (day 24 postparturition).

In order to investigate the expression of homologous sequences in other species, Northern blotting was performed with mRNA extracted from human and murine cells. First, mRNA from normal human fibroblasts and cell lines derived from different types of human tumors were probed (Fig. 3). Under high-stringency conditions, clone OCI-5 was able to detect two bands in CaCo-2, a colon tumor cell line. The predominant band had the same size as the band detected in rat cells, and the less intense band corresponded to 3.0 kb. We could not detect any homologous transcripts in the mRNA of the other cell lines, including HT-29 colon carcinoma cells. CaCo-2 cells can acquire enterocytelike characteristics when they reach confluence (25); however, the level

of OCI-5 transcripts was not dependent on culture density (data not shown). Second, we investigated the expression of the murine homolog of clone OCI-5 in F9 teratocarcinoma cells. These undifferentiated murine embryonal carcinoma cells can be induced to differentiate into parietal endoderm or visceral endodermlike cells. Figure 4 demonstrates that the undifferentiated F9 cells did not express a homologous gene, but a 2.6-kb transcript was detected after differentiation into either parietal or visceral endodermlike cells.

The relationship of OCI-5 expression to transformation of tissue-cultured cells was investigated by studying IEC-18 clones that became tumorigenic after transfection with the activated human *H-ras* oncogene (4). Unlike IEC-18 cells, which are flat and grow with a cobblestone morphology, the *H-ras*-transformed clones are spindle-shaped and adhere very poorly to plastic (4). When clone OCI-5 was hybridized against mRNA extracted from three of these clones, the 2.6-kb transcript found in the parental IEC-18 cell was undetectable, even after a very long exposure (Fig. 5A). To

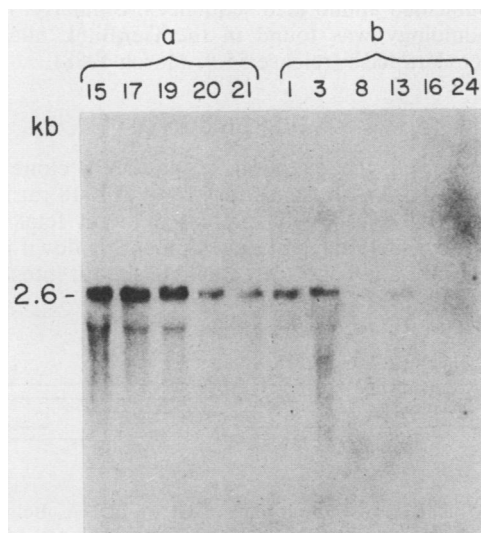


FIG. 2. Developmental regulation of clone OCI-5. Total RNA (20  $\mu$ g) was hybridized with nick-translated OCI-5. The lower band is nonspecific hybridization to the 18S rRNA. Lanes: a, days of fetal development; b, days after birth. A tubulin probe was used to confirm that the same amount of RNA was loaded into each lane.

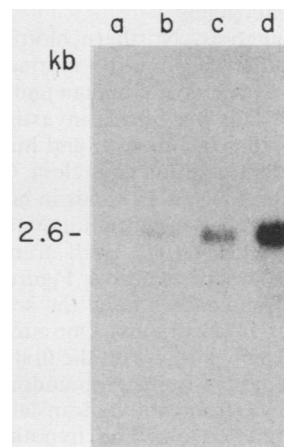


FIG. 4. Northern blot analysis of clone OCI-5 in F9 teratocarcinoma cells. mRNA (3  $\mu$ g) was hybridized with nick-translated OCI-5. Lanes: a, F9 control cells; b, parietal endodermlike cells; c, visceral endodermlike cells; d, IEC-18.

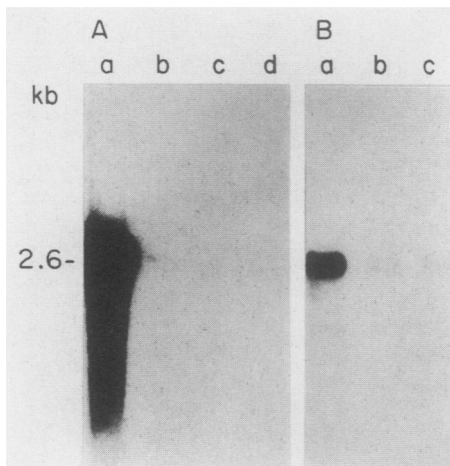


FIG. 5. Northern blot analysis of clone OCI-5 in IEC-18-transformed cells. (A) *H-ras* transformation. mRNA (10  $\mu$ g) was hybridized with nick-translated OCI-5. Lanes: a, IEC-18 control; b, c, and d, three representative transformed clones. (B) *v-src* transformation. mRNA (3  $\mu$ g) was hybridized with nick-translated OCI-5. Lanes: a, IEC-18 control; b and c, two representative transformed clones.

investigate whether this dramatic suppression of the expression of clone OCI-5 was a specific *H-ras*-related phenomenon, IEC-18 cells were also transformed with a virus containing the *v-src* gene. *v-src*-transformed clones also showed cells with a spindle-shaped morphology, although the degree of morphological change was less than that in the *H-ras* transfected clones, and flat, strongly adherent cells were also seen within individual clones (data not shown). mRNA was extracted from two representative *v-src*-infected clones, and the level of OCI-5 transcripts was determined. Figure 5B shows that clone OCI-5 was also down regulated after *v-src* infection, although a low level of expression could still be detected. Clones selected after transfection with a gene coding for resistance to G-418 showed no change in the level of expression of OCI-5 (data not shown). These results indicate that suppression of OCI-5 expression is not specifically related to activated *H-ras* expression and suggest that the down-regulation of OCI-5 expression could be related to the morphological changes produced by transformation of IEC-18 cells by oncogenes.

**Southern blot analysis.** Northern blotting of RNA from CaCo-2 human tumor cells and F9 murine teratocarcinoma cells suggested the presence of human and murine homologs to the OCI-5 gene. This was further investigated by Southern blotting of DNA from rat, mouse, and human cells. Under conditions of moderate stringency, clone OCI-5 was able to detect 10 bands in rat DNA, 11 bands in human DNA, and 7 bands in mouse DNA, after restriction with *Hind*III (Fig. 6).

**DNA sequence of clone OCI-5.** Both strands of clone OCI-5 were sequenced as described above. Figure 7 shows a partial restriction map of clone OCI-5 and the sequencing strategy that was used for both strands. One strand had an open reading frame of 1,856 bases, with the first methionine being 69 bases after the last in-frame stop codon. Figure 8 shows the sequence of that strand and its translation, starting from the first methionine codon. The hypothetical translation product would consist of 597 amino acids (69,337 daltons in molecular mass), if it is assumed that translation starts in the first ATG after the last in-frame stop codon. Two polyadenylation sites were located near the 3' end of the sequence.

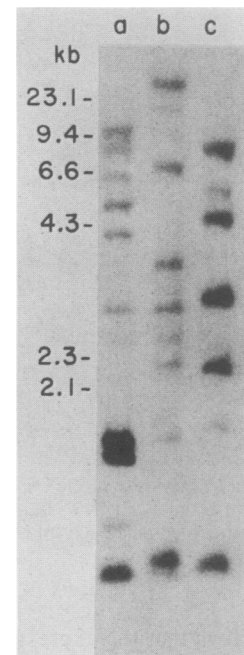


FIG. 6. Southern blot analysis of clone OCI-5 in normal mouse, human, and rat DNAs. DNA (10  $\mu$ g) was hybridized with nick-translated OCI-5. Lanes: a, mouse DNA; b, human DNA; c, rat DNA.

The predicted protein sequence included three potential glycosylation sites, but a candidate transmembrane domain (at least 20 consecutive hydrophobic residues) was not found. The amino acid hydrophobicity plot showed two mildly hydrophobic regions, one near the putative amino terminus and the other near the cysteine-rich carboxyl terminus (data not shown).

A computer search of the National Biological Research Foundation protein sequence data base (release 15.0; December 1987) detected no strong homology with any of the 7,000 published amino acid sequences. Similarly, no significant homology was found in the GenBank nucleic acid sequence data base (release 55.0; March 1988).

## DISCUSSION

We reported the isolation of a cDNA clone (OCI-5) representing a 2.6-kb transcript of the IEC-18 rat intestinal cell line. OCI-5 is highly expressed in rat fetal intestine between 15 and 19 days of development, is down regulated prior to birth, and is not detectable in the rat intestine after

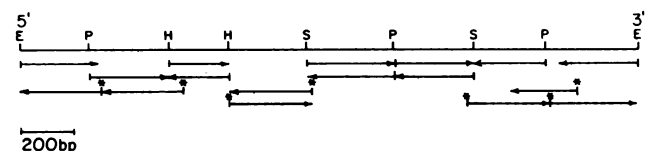


FIG. 7. Partial restriction map of OCI-5 cDNA. Below the restriction map are the fragments that were used for sequencing. Arrows indicate the direction and extent of sequence read from each fragment. Asterisks indicate the positions of the seven oligonucleotide primers that were used in the final stages of the sequence determination. Abbreviations: E, *Eco*RI; P, *Pst*I; H, *Hind*III; S, *Sst*I; bp, base pairs.



code proteins that are involved in the cytoskeletal function or in cell attachment (2). Some of the most common examples include certain forms of tropomyosin (7, 18), actin (16), collagen (1), and fibronectin (8). All these proteins are thought to be involved in the establishment of cell shape.

Much less is known about gene expression that is down regulated after transformation of epithelial cells in culture, but in a similar fashion to the situation in fibroblasts, it has been shown that transformation induces dramatic changes in the cytoskeleton and in cell attachment (20, 37). In light of these precedents and the fact that the down-regulation of clone OCI-5 in transformed IEC-18 cells was correlated with the degree of morphological change, it is tempting to speculate that the protein coded by clone OCI-5 may be involved in cytoskeletal organization or in cell attachment.

This speculation is consistent with the induction of a murine homolog to clone OCI-5 when F9 teratocarcinoma cells differentiate into parietal or visceral endodermlike cells. It has been shown that morphological changes occur during this differentiation process (35), and the induction of expression of several proteins involved in the cytoskeletal structure (15) or in cell attachment (5, 35) has been described.

Since no functional domains of the OCI-5 gene product could be identified by homology to sequences in the protein data base, further progress in the definition of the function of the protein will depend on the generation of antibodies. We have been able to express part of the OCI-5 cDNA as a fusion protein in bacteria and are now attempting to generate those antibodies.

#### ACKNOWLEDGMENTS

We are grateful to Rose Pullano and Barbara Choo for excellent technical assistance and Avril Jones for preparation of the manuscript. We thank A. Quaroni for providing the IEC cell lines.

This study was supported by a grant from the National Cancer Institute of Canada.

#### LITERATURE CITED

- Adams, S. L., J. C. Alwine, B. de Crombrughe, and I. Pastan. 1979. Use of recombinant plasmids to characterize collagen RNAs in normal and transformed chick embryo fibroblasts. *J. Biol. Chem.* **254**:4935-4938.
- Ben-Zeev, A. 1985. The cytoskeleton in cancer cells. *Biochim. Biophys. Acta* **780**:197-212.
- Boulter, C. A., and E. F. Wagner. 1988. The effects of *v-src* expression on the differentiation of embryonal carcinoma cells. *Oncogene* **2**:207-214.
- Buick, R. N., J. Filmus, and A. Quaroni. 1987. Activated *H-ras* transforms rat intestinal epithelial cells with expression of  $\alpha$ -TGF. *Exp. Cell Res.* **170**:300-309.
- Carlin, B. E., M. E. Durkin, B. Bender, R. Jaffe, and A. E. Chung. 1983. Synthesis of laminin and entactin by F9 cells induced with retinoic acid and dibutyryl cyclic AMP. *J. Biol. Chem.* **258**:7729-7737.
- Cheng, H., and C. P. Leblond. 1974. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. I. Columnar cell. *Am. J. Anat.* **141**:461-479.
- Cooper, H. L., N. Feuerstein, M. Noda, and R. H. Bassin. 1985. Suppression of tropomyosin synthesis, a common biochemical feature of oncogenesis by structurally diverse retroviral oncogenes. *Mol. Cell. Biol.* **5**:972-983.
- Fagan, J. B., M. E. Sobel, K. M. Yamada, B. de Crombrughe, and I. Pastan. 1981. Effects of transformation on fibronectin gene expression using cloned fibronectin cDNA. *J. Biol. Chem.* **256**:520-525.
- Filmus, J., J. M. Trent, R. Pullano, and R. N. Buick. 1986. A cell line from a human ovarian carcinoma with amplification of the *K-ras* gene. *Cancer Res.* **46**:5179-5182.
- Hedrick, S. M., D. I. Cohen, E. A. Nielsen, and M. M. Davis. 1984. Isolation of cDNA clones encoding T cell-specific membrane-associated proteins. *Nature (London)* **308**:149-153.
- Hermos, J. A., M. Mathan, and J. S. Trier. 1971. DNA synthesis and proliferation by villous epithelial cells in fetal rats. *J. Cell Biol.* **50**:255-258.
- Hogan, B., F. Constantini, and E. Lacy (ed.). 1986. *Manipulating the mouse embryo*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Kedinger, M., P. M. Simon-Assmann, B. Lacroix, A. Marxer, H. P. Hauri, and K. Haffen. 1986. Fetal gut mesenchyme induces differentiation of cultured intestinal endodermal and crypt cells. *Dev. Biol.* **113**:474-483.
- Kurokawa, M., K. Lynch, and D. K. Podolsky. 1987. Effects of growth factors on an intestinal epithelial cell line: transforming growth factor  $\beta$  inhibits proliferation and stimulates differentiation. *Biochem. Biophys. Res. Commun.* **142**:775-782.
- Lampron, C., and A. Royal. 1987. Expression of new proteins of the intermediate filament protein family in differentiation F9 embryonal carcinoma cell cytoskeleton. *J. Biol. Chem.* **262**:4893-4898.
- Leavitt, J., P. Gunning, L. Kedes, and R. Sariwalla. 1985. Smooth muscle  $\alpha$ -actin is a transformation-sensitive marker for mouse NIH3T3 and rat-2 cells. *Nature (London)* **316**:840-842.
- Mathan, M., P. C. Moxey, and J. S. Trier. 1976. Morphogenesis of fetal rat duodenal villi. *Am. J. Anat.* **146**:73-92.
- Matsumura, F., and S. Matsumura. 1986. Tropomyosin in cell transformation. *Cancer Rev.* **6**:21-39.
- Messing, J., and J. Vieira. 1982. A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. *Gene* **19**:269-276.
- Miyazaki, K., Y. Ashida, Y. Kihira, K. Mashima, J. Yamashita, and T. Horio. 1987. Transformation of rat liver cell line by Rous sarcoma virus causes loss of cell surface fibronectin, accompanied by secretion of metallo-proteinase that preferentially digests the fibronectin. *J. Biochem.* **102**:569-582.
- Okayama, H., and P. Berg. 1982. High-efficiency cloning of full-length cDNA. *Mol. Cell. Biol.* **2**:161-169.
- Pathak, S., M. J. Siciliano, R. Cailleau, C. L. Wiseman, and T. L. Hsu. 1979. A human breast adenocarcinoma with chromosome and isoenzyme markers similar to those of the HeLa line. *JNCI* **62**:263-271.
- Pfreundschuh, M., H. Shiku, T. Takahashi, R. Veda, J. Ransohoff, H. F. Oettgen, and L. J. Old. 1978. Serological analysis of cell surface antigens of malignant human brain tumors. *Proc. Natl. Acad. Sci. USA* **75**:5122-5126.
- Pinto, M., M. D. Appay, P. M. Simon-Assman, G. Chevalier, N. Dracopoli, J. Fogh, and A. Zweibaum. 1982. Enterocytic differentiation of cultured human colon cancer cells by replacement of glucose by galactose in the medium. *Biol. Cell* **44**:193-196.
- Pinto, M., S. Robine-Leon, M. D. Appay, M. Kedinger, N. Triadou, E. Dussaulx, B. Lacroix, P. Simon-Assman, K. Haffen, J. Fogh, and A. Zweibaum. 1983. Enterocyte-like differentiation and polarization of the human colon carcinoma cell line CaCo-2 in culture. *Biol. Cell* **47**:323-330.
- Potten, C. S., and J. H. Hendry. 1983. In C. S. Potten (ed.), *Stem cells: their identification and characterization*, p. 155-199. Churchill Livingstone, Ltd., Edinburgh.
- Quaroni, A. 1985. Crypt cell development in newborn rat small intestine. *J. Cell Biol.* **100**:1601-1610.
- Quaroni, A. 1985. Development of fetal rat intestine in organ and monolayer culture. *J. Cell Biol.* **100**:1611-1622.
- Quaroni, A. 1986. Crypt cell antigen expression in human colon tumor cell lines: analysis with a panel of monoclonal antibodies to CaCo-2 luminal membrane components. *JNCI* **76**:571-585.
- Quaroni, A. 1986. Fetal characteristics of small intestinal crypt cells. *Proc. Natl. Acad. Sci. USA* **83**:1723-1727.
- Quaroni, A., and K. J. Isselbacher. 1981. Cytotoxic effects and metabolism of benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene in duodenal and ileal epithelial cell cultures. *JNCI* **67**:1353-1362.
- Quaroni, A., S. Wands, R. L. Trelstad, and K. J. Isselbacher. 1979. Epithelioid cell cultures from rat small intestine. *J. Cell*

- Biol. **80**:248–265.
33. Sanger, F., A. R. Coulson, B. G. Barrell, A. J. Smith, and B. A. Roe. 1980. Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. *J. Mol. Biol.* **143**:161–178.
  34. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463–5467.
  35. Strickland, S., and V. Mahdavi. 1978. The induction of differentiation in teratocarcinoma stem cells by retinoic acid. *Cell* **15**:393–403.
  36. Topp, W. C. 1981. Normal rat cell lines deficient in nuclear thymidine kinase. *Virology* **113**:408–411.
  37. Warren, S. L., L. M. Handel, and W. J. Nelson. 1988. Elevated expression of pp60<sup>c-src</sup> alters a selective morphogenetic property of epithelial cells in vitro without a mitogenic effect. *Mol. Cell. Biol.* **8**:632–646.
  38. Weiser, M. M. 1973. Intestinal epithelial cell surface membrane glycoprotein synthesis. II. Glycosyltransferases and endogenous acceptors of the undifferentiated cell surface membrane. *J. Biol. Chem.* **248**:2542–2548.