MOLECULAR AND CELLULAR BIOLOGY, June 1983, p. 1062–1069
0270-7306/83/061062-08$02.00/0

Vol. 3, No. 6

Transcription of c-*onc* Genes c-*ras*^{Ki} and c-*fms* During Mouse Development

ROLF MÜLLER,[1]†* DENNIS J. SLAMON,[2] EILEEN D. ADAMSON,[3] JOANNE M. TREMBLAY,[1] DAGMAR MÜLLER,[1] MARTIN J. CLINE,[2] AND INDER M. VERMA[1]

Molecular Biology and Virology Laboratory, The Salk Institute, San Diego, California 92138[1]; Division of Hematology/Oncology, Department of Medicine, University of California Medical Center, Los Angeles, California 90024[2]; and Cancer Research Center, La Jolla Cancer Research Foundation, La Jolla, California 92037[3]

Received 22 November 1982/Accepted 22 March 1983

We investigated the expression of cellular sequences c-*ras*^{Ki} and c-*fms*, which are homologous to the oncogenes of Kirsten rat sarcoma virus and the McDonough strain of feline sarcoma virus, during murine development and in a variety of mouse tissues. The c-*ras*^{Ki} gene was found to be transcribed into two mRNA species of approximately 2.0 and 4.4 kilobases, whereas a single c-*fms*-related transcript of approximately 3.7 kilobases was identified. The c-*ras*^{Ki} gene appeared to be expressed ubiquitously, since similar levels of transcripts were observed in embryos, fetuses, extraembryonal structures, and a variety of postnatal tissues. In contrast, significant expression of c-*fms* was found to be confined to the placenta and extraembryonal membranes (i.e., combined yolk sac and amnion). The concentration of c-*fms* transcripts in the placenta increased approximately 15-fold (relative to day-7 to day-9 conceptuses) during development before reaching a plateau at day 14 to 15 of gestation. The time course of c-*fms* expression in the extraembryonal membranes appeared to parallel the stage-specific pattern observed in the placenta. The level of c-*fms* transcripts in the extraembryonal tissues reached a level which was approximately 20- to 50-fold greater than that in the fetus. These findings suggest that the c-*fms* gene product may play a role in differentiation of extraembryonal structures or in transport processes occurring in these tissues. Our results indicate that the c-*onc* genes analyzed in the present study exert essentially different functions during mouse development.

The acutely oncogenic retroviruses contain sequences in their genomes which are required for induction of neoplasia in vivo and for morphological and malignant transformation of cultured cells in vitro (3, 50). These sequences, termed viral oncogenes (v-*onc*), apparently originated from the normal vertebrate genome (3, 50). The precise mechanism of acquisition of these cellular sequences remains obscure, but it presumably involves recombination between the genome of a replication-competent retrovirus and the host genome (3, 50). To date, 16 different cellular homologs of retroviral oncogenes have been identified (7, 12–14, 16–22, 26, 33–35, 37, 39–42, 44, 45, 48, 50, 52, 53) and have been termed cellular oncogenes (c-*onc*). The structures of some v-*onc* genes and their encoded proteins have been shown to be closely related to those of their cellular homologs (3, 50). At least two c-*onc* genes have indeed been found to exhibit transforming potential in vitro when linked to a retroviral long terminal repeat (4, 13).

The function of c-*onc*-encoded proteins has been a subject of intense interest and speculation (2, 3, 11, 18, 24, 31, 50). Transcriptional activity of several c-*onc* genes has been observed in normal cells or tissues. The first such finding was made with c-*src* (cellular homolog of the oncogene of Rous sarcoma virus (34, 43, 49), which was followed by a number of reports on transcription of other c-*onc* genes, mainly of avian origin (8, 16, 23, 37, 40, 48). In some instances, c-*onc*-encoded proteins have also been identified (10, 28, 29, 32, 38, 51).

Since v-*onc* gene products are known to interfere with normal cell differentiation and the control of cell proliferation, it is a widely accepted hypothesis that their cellular counterparts may play a physiological role in such processes (2, 3, 11, 18, 24, 31, 50). To study the function of c-*onc* genes in murine development and normal cell metabolism, we have undertaken a systematic investigation of c-*onc* transcription during

† Present address: European Molecular Biology Laboratory, D-6900 Heidelberg, Federal Republic of Germany.

pre- and postnatal development of mice. We have recently reported (31) that the cellular homolog c-*fos* of the oncogene of FBJ murine osteosarcoma virus is expressed at elevated levels in late-gestation fetuses, whereas the greatest concentrations of transcripts from the cellular counterpart c-*abl* of Abelson murine leukemia virus were found in midgestation fetuses. In addition, elevated levels of expression of both c-*onc* genes were observed in a restricted number of tissues: c-*fos* in placenta as well as in bone and skin tissues from newborn mice, and c-*abl* in adult testes. In contrast, the cellular homolog(s) (c-ras^{Ha}) of the oncogene of Harvey rat sarcoma virus (HaSV) was found to be transcriptionally active at relatively high levels in embryos and fetuses throughout mouse prenatal development and in a variety of extraembryonal and postnatal tissues. In the present study we analyzed the expression of the cellular homologs c-ras^{Ki} and c-*fms* of the oncogenes of Kirsten rat sarcoma virus (KiSV) and the McDonough strain of feline sarcoma virus (FeSV) during development of the mouse fetus, placenta, and extraembryonal membranes as well as in postnatal tissues. The characteristics of the oncogenes analyzed in this study and the v-*onc*-specific fragments utilized are summarized in Table 1.

MATERIALS AND METHODS

Animal experiments. Random-bred Swiss Webster mice were mated, and the day of genital plug was designated as day 1 of gestation. Day-7 to day-9 embryos represented the entire conceptus as dissected from the uterine wall, including all extraembryonal tissues, such as membranes and cells which give rise to the placenta (e.g., trophoblast and decidua). At all later stages of gestation, the embryo proper was dissected free of extraembryonal tissues.

Cell culture. Cells were cultured in Dulbecco-Vogt modified Eagle minimum essential medium supplemented with antibiotics and 10% fetal calf serum. The Ki-3T3 cell line was kindly provided by B. Sefton (The Salk Institute, San Diego, Calif.).

Isolation of RNA. All solutions, except for buffers containing guanidine thiocyanate or guanidine hydrochloride, were made RNase free by treatment with 0.02% diethyl pyrocarbonate and then autoclaved. All glassware was baked for 4 h at 180°C. Total RNA was isolated from mouse tissues or cultured cells according to the procedure described by Chirgwin et al. (9). Tissues or cells were vigorously homogenized (using either a motor-driven tissue homogenizer or a Dounce homogenizer), followed by forcing the homogenate several times through a 21-gauge needle attached to a 10-ml syringe. The homogenization buffer contained 4 M guanidine thiocyanate (Eastman Kodak), 0.5% lauroylsarcosine (Sigma Chemical Co.), 0.1 M 2-mercaptoethanol, 0.025 M sodium citrate (pH 7.0), and 0.1% Antifoam A (Sigma), adjusted to pH 7.0 (16 ml of buffer per g of tissue or cell pellet and 32 ml of buffer per g of spleen tissue). Homogenate (8 ml) was layered on top of a 3.5-ml cushion of 5.7 M cesium chloride in 0.025 M sodium acetate (pH 5.0) and centrifuged for 16 to 20 h at 36,000 rpm and 20°C in a Beckman SW41 rotor. The supernatant was withdrawn, the tube was rinsed with ethanol, and the RNA pellet was suspended in 0.4 ml of water. After addition of 2.6 ml of a buffer containing 7.5 M guanidine hydrochloride (Sigma), 0.005 M dithiothreitol, and 0.025 M sodium citrate (pH 7.0), the pH was lowered to approximately 5.0 by addition of 75 μl of 1 M acetic acid. The RNA was then precipitated by addition of 0.5 volumes of cold absolute ethanol. After at least 4 h at −20°C, the RNA precipitate was recovered by centrifugation for 15 min at 10,000 × *g*, and the pellet was extracted three times with 0.5 ml of water. The extracted RNA solutions were pooled, made 0.2 M in sodium acetate, mixed with 2 to 2.5 volumes of absolute ethanol, and kept at −20°C overnight. The precipitated RNA was recovered by centrifugation for 15 min at 10,000 × *g* and dissolved in 1 ml of water, and the concentration was determined spectrophotometrically, based on an optical density at 260 nm (OD_{260}) of 24 for 1 mg of RNA per ml.

Poly(A)$^+$ selection of RNA. Total RNA was enriched for polyadenylic acid [poly(A)]-containing [poly(A)$^+$]RNA by one cycle of affinity chromatography on oligodeoxythymidylic acid-cellulose (1). Briefly, 2-ml syringes were filled with approximately 0.5 ml of oligodeoxythymidylic acid-cellulose gel (P-L Biochemicals, Inc., type 7), preswollen in low-salt buffer (0.1% sodium dodecyl sulfate, 0.01 M Tris-hydrochloride [pH 7.4], and 0.005 M EDTA). The gel was washed extensively with the same buffer and equili-

TABLE 1. Oncogenes analyzed

Retrovirus	Disease induced	Oncogene	Viral oncogene product	Cellular oncogene product	Fragment used as probe (kbp)	Reference
Murine						
HaSV	Erythroleukemia, lymphoma, sarcoma	ras^{Ha}	P21	P21	*Bgl*I-*Sal*I (0.46)	16
KiSV	Erythroleukemia, lymphoma, sarcoma	ras^{Ki}	P21	P21	*Hin*cII-*Hin*cII (0.5)	17
Feline						
FeSV McDonough strain	Multicentric sarcomas	*fms*	P170	?	*Pst*I-*Pst*I (~1.6)	14

brated with high-salt buffer (0.5 M NaCl, 0.1% sodium dodecyl sulfate, 0.01 M Tris-hydrochloride [pH. 7.4], and 0.005 M EDTA). RNA was boiled for 2 to 3 min, quick-cooled, and applied at a concentration of 0.1 to 0.2 mg/ml of high-salt buffer at the natural flow rate. The eluate was reapplied, and columns were washed with high-salt buffer until the OD_{260} of the eluate was less than 0.02. Bound RNA was eluted with 2 ml of low-salt buffer and recovered by ethanol precipitation. Enrichment for poly(A)$^+$ RNA was approximately 10- to 20-fold.

Dot-blot analysis. Poly(A)$^+$ RNA was dissolved in water, boiled, and quick-cooled on ice, and 1 to 2 μg (1 μl) was applied to sheets of nitrocellulose paper, which had previously been equilibrated with 20× SSC (1× SSC: 0.15 M NaCl and 0.015 M sodium citrate) and air dried. After baking for 4 to 20 h at 80°C, the blots were prehybridized for 4 h at 45°C in a buffer containing 0.75 M NaCl, 0.05 M sodium phosphate (pH 7.5), 0.005 M EDTA, 0.2% sodium dodecyl sulfate, 10 mg of glycine per ml, 5× Denhardt reagent (1× Denhardt reagent: 0.02% each of Ficoll, bovine serum albumin, and polyvinylpyrrolidone), 0.25 mg of denatured herring DNA per ml, and 50% formamide. Subsequently, the blots were hybridized for approximately 20 h at 45°C with 10^6 cpm of nick-translated probe per ml of hybridization buffer (same composition as prehybridization buffer except that the concentration of Denhardt reagent was decreased to 1×). The cloned oncogene fragments (see Table 1), purified from vector sequences by preparative agarose gel electrophoresis, were nick translated (36) in the presence of [α-^{32}P]dCTP (3,200 Ci/mmol) to specific radioactivities of approximately 10^9 cpm of DNA per μg. The *fms*-specific probe was obtained by subcloning a 1.5-kilobase (kb) *Pst*I fragment from the McDonough strain of FeSV (SM FeSV) (14) into the *Pst*I site of plasmid pBR322. After hybridization, the blots were washed three times in 1× SSC at 50°C for a total of 2 h and exposed to X-ray films with intensifying screens at −70°C.

Agarose gel electrophoresis and blotting of gels. Poly(A)$^+$ RNA (20 μg) was denatured in 1 M glyoxal and 20 mM sodium phosphate (pH 7.0) for 1 h at 50°C. The denatured RNA was separated on horizontal gels consisting of 1.1% agarose in 10 mM sodium phosphate buffer (pH 7.0) for about 20 h at 35 V. Thereafter the RNA was blotted onto nitrocellulose paper with 20× SSC (46). Blots were baked for 3 h at 80°C and prehybridized, hybridized, and washed as described above for dot blots, except that the hybridization was carried out in the presence of 5 × 10^6 cpm of nick-translated probe per ml for 60 h at 45°C.

RESULTS

Strategy. Our strategy involved isolation of fetuses, placentas, and extraembryonal membranes from random-bred Swiss Webster mice between days 7 and 18 of gestation. The extracted total RNA was enriched for poly(A)$^+$ mRNA by affinity chromatography on oligodeoxythymidylic acid-cellulose and analyzed by a dot-blot technique and agarose gel electrophoresis followed by Northern blotting for hybridization to v-*onc*-specific probes. Dot-blot autoradiograms were quantitatively evaluated by scanning with a densitometer. The variation of results obtained in independent experiments was in the range of two-fold. We included an alpha-fetoprotein-specific probe in our studies as a stage- and tissue-specific positive control. Our findings (see Fig. 1 and 3a) are in good agreement with published data on protein and RNA expression (15, 31). The absence of detectable hybridization of a v-*mos*-specific probe (oncogene of Moloney murine sarcoma virus; *Xba*I-*Hin*dIII fragment) (26) to any RNA sample served to show that nonspecific hybridization is negligible in the dot-blot technique used in this study (data not shown) (31).

Expression of c-*ras*^{Ha} and c-*ras*^{Ki}. HaSV and KiSV cause erythroblastosis, extrathymic lymphomas, and sarcomas in rodents (25, 27). The oncogenes (v-*ras*^{Ha} and v-*ras*^{Ki}) of both viruses originated from normal rat cellular sequences and encode 21K proteins (P21) which are biochemically, immunologically, and functionally related to each other (6, 13, 16–18). Despite the kinship of the *ras*-encoded proteins, DNA probes specific for v-*ras*^{Ha} or v-*ras*^{Ki} sequences have been shown to hybridize with different restriction fragments of mammalian genomic DNA, which indicates that the cellular homologs (c-*ras*^{Ha} and c-*ras*^{Ki}, respectively) represent independent cellular genes within the *ras* gene family (18). Proteins (P21) encoded by c-*ras* have been described in a variety of mammalian cells and tissues (28, 38). We have previously shown that the c-*ras*^{Ha} gene is transcriptionally active at relatively high but constant levels in embryos/fetuses and extraembryonal tissues at all stages of murine development examined (days 6 to 18) (31). Therefore, the c-*ras*^{Ha} gene was included in the present study (see Fig. 1 and 2) as an internal control. This served to show that stage- or tissue-specific patterns of expression observed with other c-*onc* genes are not due to variations in the poly(A)$^+$ RNA concentrations of the samples applied to nitrocellulose filters for dot-blot analysis.

As shown in Fig. 1, transcriptional activity of the c-*ras*^{Ki} gene could be detected throughout prenatal development, although approximately 50 to 75% lower concentrations of c-*ras*^{Ki}-related transcripts were observed in day-18 fetuses relative to midgestation embryos (average values of three independent experiments utilizing different batches of RNA; Fig. 1 and 2 and unpublished data). High levels of c-*ras*^{Ki} expression were also observed in day-12 to -18 placentas and extraembryonal membranes (Fig. 3a). Transcriptional activity of c-*ras*^{Ki} appears to be similar in most postnatal tissues examined in the present study (Fig. 3b). Agarose gel electrophoresis followed by transfer of the RNA to nitro-

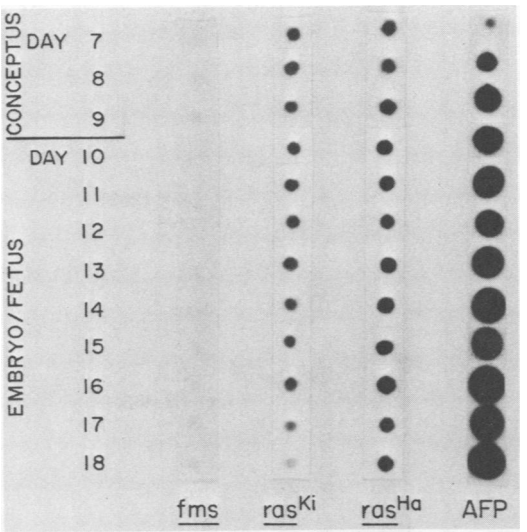

FIG. 1. Transcription of c-*onc* genes during mouse prenatal development. Hybridization of v-*onc* and AFP-specific probes to poly(A)$^+$ RNA from day-7 to day-9 conceptuses and day-10 to day-18 embryos and fetuses, respectively. Dot-blot analysis was performed as described in the text. Probes specific for v-*ras*^{Ha} and AFP were included as internal controls. Exposure times were selected so that signals with mouse DNA were similar for all probes.

cellulose paper and hybridization to the v-*ras*^{Ki}-specific probe revealed two c-*ras*^{Ki}-related transcripts of 2.0 and 4.4 kb in day-12 membranes, day-12 placenta, and in day-17 fetuses (Fig. 4). The same transcripts were detected in postnatal peritoneum and heart (Fig. 4). Similarly sized transcripts related to c-*ras*^{Ki} have recently been described in cell lines of mouse origin (16).

Expression of c-*fms*. FeSV causes multicentric fibrosarcomas in cats. The oncogene of the McDonough strain of FeSV (30) has been designated v-*fms*, and its normal cellular homolog (c-*fms*) has been detected in normal vertebrate DNA (14). The v-*fms* gene product has been identified as a 170-kilodalton *gag-onc* fusion protein (P170) (14). No c-*fms*-encoded transcript or protein has yet been described.

Analysis of c-*fms* expression revealed a highly specific pattern of transcriptional activity (Fig. 1, 3, 5, and 6). Low levels of c-*fms* expression were detected in embyros and fetuses at all stages of development (Fig. 1 and 3a) and in all postnatal tissues investigated (Fig. 3b). In contrast, the abundance of c-*fms* transcripts increased gradually during placental development and reached a maximum at day 14 to 15 of gestation (Fig. 3a and 5). In day-15 to -18 placentas the concentration of c-*fms* transcripts was found to be approximately 15-fold greater than in day-7 to -9 conceptuses (which consist largely of tissues participating in the formation of the placenta). The pattern of c-*fms* expression during development of the extraembryonal membranes closely resembled the stage-specific pattern of expression observed during development of the placenta (Fig. 3a and 5). An approximately sixfold higher level of c-*fms* transcripts was observed in the extraembryonal membranes at day 18 relative to day 12 of gestation. Transcripts from the c-*fms* gene were found at two- to threefold higher levels in late-gestation placentas than in extraembryonal membranes of the same developmental stage (results of three independent experiments). Agarose gel electrophoresis revealed a single transcript from the c-*fms* gene of approximately 3.7 kb (Fig. 6). This transcript was detected at gradually increasing concentrations during development of the placenta and in day-17 membranes (Fig. 6). No c-*fms*-related transcript was found in the day-17 fetus (Fig. 6). These findings confirm the results of the dot-blot analyses (Fig. 3a and 5).

Quantification of c-*onc* expression. To estimate the average level of c-*ras*^{Ki} expression during mouse prenatal development, transcriptional activity in day-11 embryos was compared with expression of v-*ras*^{Ki} in a cell line transformed by KiSV (Ki-3T3). The level of v-*onc* expression in cells transformed by RNA tumor viruses is usually in the range of 0.1 to 2% of the total poly(A)$^+$ RNA (e.g., references 23 and 40). Various amounts of poly(A)$^+$ RNA from the

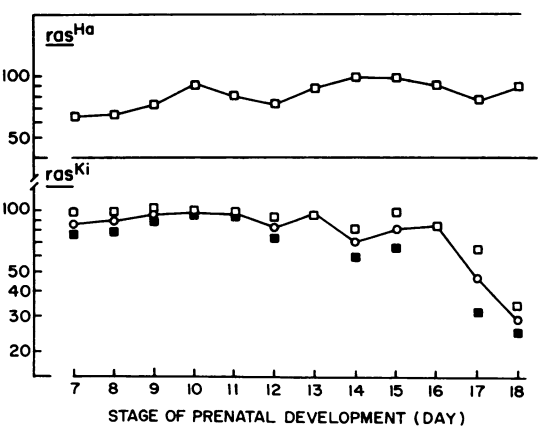

FIG. 2. Relative levels of c-*ras*^{Ki} and c-*ras*^{Ha} transcripts during embryonal/fetal development plotted against the stage of prenatal development. Values were normalized to 100 for the highest level of expression in the case of each c-*onc* gene. Symbols: □, quantification by densitometer of the dot-blot autoradiograms shown in Fig. 1 (*ras*^{Ki} and *ras*^{Ha}); ■, values from a second experiment with independently isolated RNA samples (*ras*^{Ki}); ○, average values of both experiments (*ras*^{Ki}).

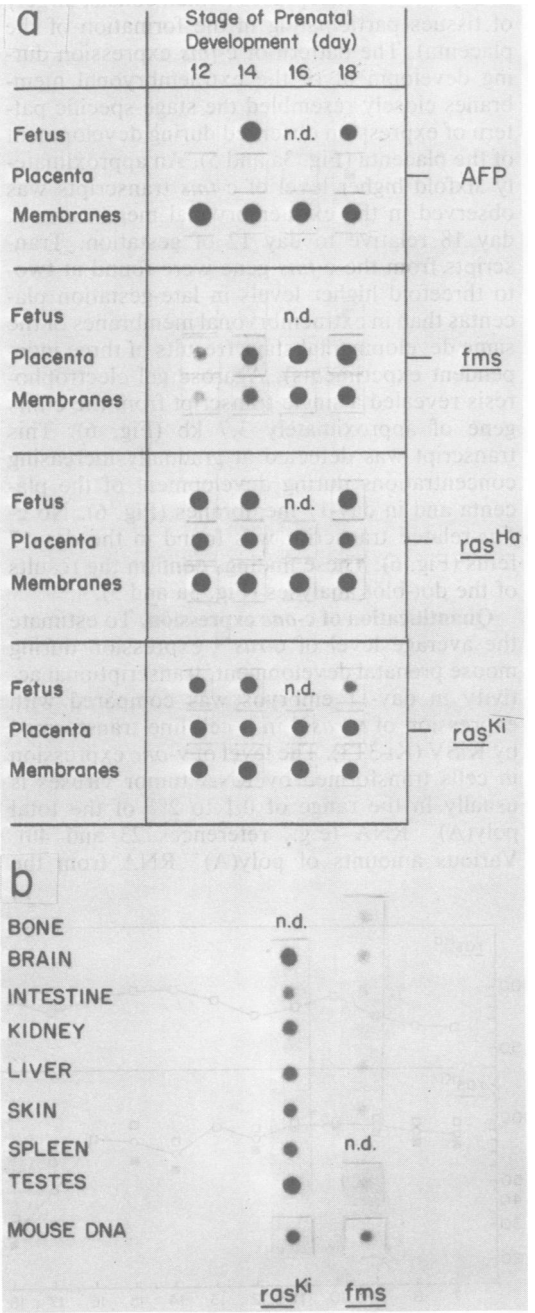

FIG. 3. Expression of c-*onc* genes in pre- and postnatal mouse tissues. (a) Transcripts from c-*onc* genes in extraembryonal tissues compared with fetuses. Hybridization of AFP and v-*onc*-specific probes to 1 μg of poly(A)$^+$ RNA from day-12, -14, -16, and -18 fetuses, placentas, and extraembryonal membranes. Exposure times were 1.5 h for alpha-fetoprotein (AFP) and 30 h for *ras*^{Ha} and *ras*^{Ki}. (b) Transcripts from c-*onc* genes in postnatal mouse tissues. Hybridization of v-*onc*-specific probes to 1 μg of poly(A)$^+$ RNA from various tissues from 8-day-old mice. "Bone" (ribs, sternum, and vertebra, containing bone marrow and some adherent connective tissue) and liver were from 1-day-old animals, and testes were from adult mice. Exposure time was 48 h. n.d., Not done.

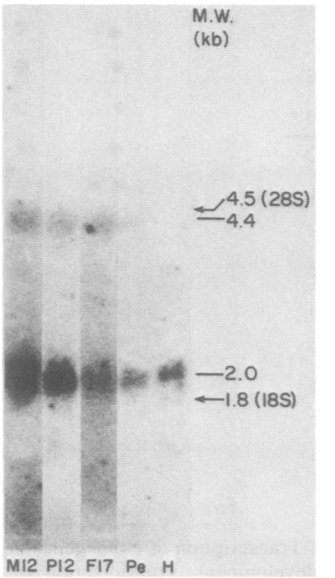

FIG. 4. Transcripts from the c-*ras*^{Ki} gene in pre- and postnatal mouse tissues. Poly(A)$^+$ RNA was analyzed by agarose gel electrophoresis followed by blotting onto nitrocellulose paper and hybridization to the v-*ras*^{Ki}-specific probe. Autoradiograms show c-*ras*^{Ki} transcripts in day-12 membranes (M12); day-12 placenta (P12); day-17 fetus (F17); postnatal (day-8) peritoneum (body wall) (Pe); and heart (H). Exposure time was 60 h. MW, Molecular size in kilobases (kb).

virus-transformed cell line were applied to nitrocellulose paper along with a constant amount of poly(A)$^+$ RNA from day-11 embryos and were hybridized to the v-*ras*^{Ki} specific probe. Figure 7 shows that the average level of c-*ras*^{Ki} expression in the total embryo is approximately 30-fold lower than that of v-*ras*^{Ki} in the KiSV-transformed cell line. We have previously shown (31) that v-*ras*^{Ha} is expressed at about 10-fold higher levels in an HaSV-transformed cell line compared with its cellular counterpart in day-11 embryos. The concentration of KiSV transcripts in the Ki-3T3 cell line is approximately threefold higher than the concentration of HaSV transcripts in the previously investigated cell line (31). We therefore conclude that c-*ras*^{Ki} is expressed in day-11 embryos at average levels similar to c-*ras*^{Ha} and c-*abl* and represents 0.01 to 0.05% of the total cellular poly(A)$^+$ RNA. If c-*ras*^{Ki} and c-*fms* are transcriptionally active at significantly elevated levels in a restricted number of cells, the level of expression in these

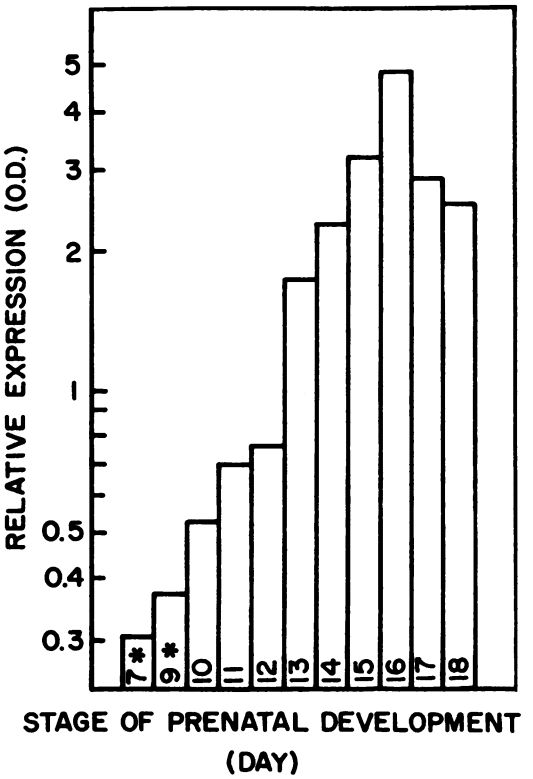

FIG. 5. Relative levels of c-*fms* transcripts during development of the placenta plotted against the stage of prenatal development. Total RNA (5 μg) from different stages of placental development was analyzed by dot blot for c-*fms* expression, and the autoradiogram was quantitatively evaluated by scanning with a densitometer. *, Total conceptuses.

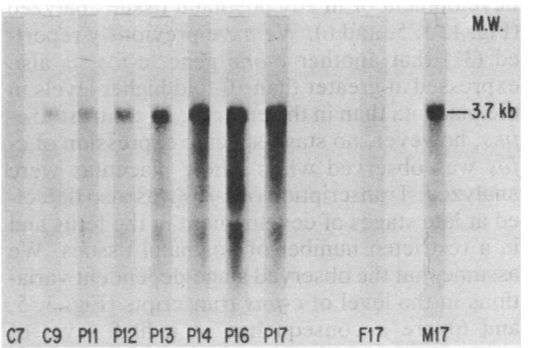

FIG. 6. Transcripts from the c-*fms* gene in prenatal mouse tissues. Poly(A)$^+$ RNA was separated by agarose gel electrophoresis, transferred to nitrocellulose paper, and hybridized to the v-*fms*-specific probe. Autoradiograms show c-*fms* transcripts in day-7 and -9 conceptuses (C7 and C9); day-11 to -17 placentas (P11 through P17); day-17 fetus (F17); and day-17 membranes (M17). Sizes (MW) are relative to 18S/28S rRNA (see Fig. 4). Exposure time was 60 h. The same filter was hybridized to the v-*ras*^{Ha}-specific probe, and no variations in the level of expression were observed throughout placental development and in day-17 fetuses and membranes.

particular cells could be considerably higher than the determined average value.

DISCUSSION

Despite an extensive knowledge of the structure of several c-*onc* genes, their role in normal cellular metabolism remains largely obscure. In the present study we demonstrated that the cellular homologs of two retroviral oncogenes, *ras*^{Ki} and *fms*, are transcriptionally active during mouse development. The pattern of c-*fms* expression showed a particularly remarkable specificity (Fig. 1, 3, 5, and 6).

The oncogenes of HaSV and KiSV (v-*ras*^{Ha} and v-*ras*^{Ki}) originated from divergent members of a family of normal vertebrate genes (18). Their cellular homologs, c-*ras*^{Ha} and c-*ras*^{Ki}, both appear to be expressed ubiquitously in mouse embryos, fetuses, placentas, and extraembryonal membranes as well as in postnatal tissues (Fig. 1 through 4). In contrast to these rather unspecific patterns of expression, the c-*fms* gene was found to be transcriptionally active at elevated levels specifically in the placenta and the extraembryonal membranes (Fig. 1, 3, 5, and 6). Significant expression of c-*fms* could not be detected in embryos or fetuses at any stage of

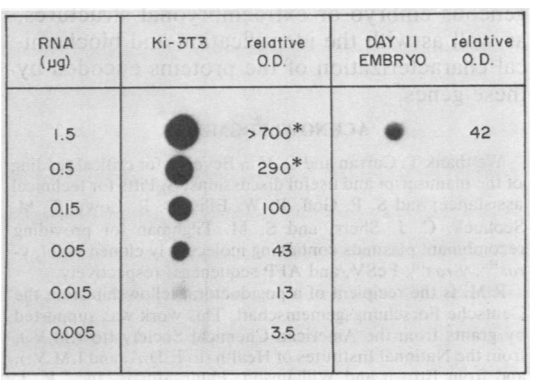

FIG. 7. Average level of c-*ras*^{Ki} expression in embryos compared with a KiSV-transformed cell line. Poly(A)$^+$ RNA (1.5 μg) from embryos at day 11 of gestation and various amounts of poly(A)$^+$ RNA from the Ki-3T3 cell line were spotted on nitrocellulose paper and hybridized to the v-*ras*^{Ki}-specific probes. Relative optical densities (O.D.) indicate the values measured by density scanning of the autoradiogram, normalized to 100 for 0.15 μg of RNA. *, Optical density readings obtained from autoradiogram exposed for only 6 h (other values, 24-h exposure).

development or in any postnatal tissue analyzed (Fig. 1, 3, 5, and 6). We have previously reported (31) that another c-*onc* gene, c-*fos*, is also expressed at greater than 10-fold higher levels in the placenta than in the embryo. In contrast to c-*fms*, however, no stage-specific expression of c-*fos* was observed when whole placentas were analyzed. Transcription of c-*fos* was also detected at late stages of development in the fetus and in a restricted number of postnatal tissues. We assume that the observed stage-dependent variations in the level of c-*fms* transcripts (Fig. 3, 5, and 6) are a consequence of a high level of expression in a limited number of cell types present in the placenta and the extraembryonal membranes, whose number increases as gestation proceeds.

In this and other studies (31, R. Müller, I. M. Verma, and E. D. Adamson, EMBO J., in press) we have demonstrated that cellular sequences homologous to retroviral oncogenes are transcriptionally active during development of the mouse embryo and fetus and extraembryonal structures as well as in postnatal tissues. In the case of most c-*onc* genes analyzed, stage, or tissue-specific patterns of expression or both were observed. Our findings of a modulated control of c-*onc* gene expression, taken together with the high evolutionary conservation of these genes (3, 41, 50), strongly suggest that c-*onc*-encoded proteins may indeed serve important physiological functions such as a role in growth and development. Our further studies will now be concerned with the exact localization by in situ hybridization (5) of those cell types that exhibit enhanced c-*onc* expression in the heterogeneous embryo or extraembryonal structures, as well as with the identification and biochemical characterization of the proteins encoded by these genes.

ACKNOWLEDGMENTS

We thank T. Curran and C. Van Beveren for critical reading of the manuscript and useful discussions; S. Pitts for technical assistance; and S. P. Goff, R. W. Ellis, D. R. Lowy, E. M. Scolnick, C. J. Sherr, and S. M. Tilghman for providing recombinant plasmids containing molecularly cloned v-*abl*, v-*ras*^{Ha}, v-*ras*^{Ki}, FeSV, and AFP sequences, respectively.

R.M. is the recipient of a postdoctoral fellowship from the Deutsche Forschungsgemeinschaft. This work was supported by grants from the American Chemical Society (to I.M.V.), from the National Institutes of Health (to E.D.A. and I.M.V.), and from Brown and Williamson, Philip-Morris, Inc., R. J. Reynolds, and the U.S. Tobacco Co. (to M.J.C.).

LITERATURE CITED

1. **Aviv, H., and P. Leder.** 1972. Purification of biologically active globin messenger RNA by chromatography on oligo-thymidylic acid-cellulose. Proc. Natl. Acad. Sci. U.S.A. **69**:1408–1412.
2. **Bishop, J. M.** 1981. Retrovirus oncogenes, p. 515–524, *In* D. D. Brown (ed.), Developmental biology using purified genes. Academic Press, Inc., New York.
3. **Bishop, J. M.** 1981. Enemies within: the genesis of retroviral oncogenes. Cell **23**:5–6.
4. **Blair, D. G., M. Oskarsson, T. G. Wood, W. L. McClements, P. J. Fischinger, and G. G. Vande Woude.** 1981. Activation of the transforming potential of a normal cell sequence: a molecular model for oncogenesis. Science **212**:941–943.
5. **Brahic, M., and A. T. Haase.** 1978. Detection of viral sequences of low reiteration frequency by *in situ* hybridization. Proc. Natl. Acad. Sci. U.S.A. **75**:6125–6129.
6. **Chattopadhyay, S. K., E. H. Chang, M. L. Lander, R. W. Ellis, E. M. Scolnick, and D. R. Lowy.** 1982. Amplification and rearrangement of *onc* genes in mammalian species. Nature (London) **296**:361–363.
7. **Chen, I. S. Y., T. W. Mak, J. J. O'Rear, and H. M. Temin.** 1982. Characterization of reticuloendotheliosis virus strain T DNA and isolation of a novel variant of reticuloendotheliosis virus strain T by molecular cloning. J. Virol **40**:800–811.
8. **Chen, J. H.** 1980. Expression of endogenous avian myeloblastosis virus information in different chicken cells. J. Virol. **36**:162–170.
9. **Chirgwin, J. M., A. E. Przybala, R. Y. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**:5294–5299.
10. **Collett, M. S., J. S. Brugge, and R. L. Erikson.** 1978. Characterization of a normal avian cell protein related to the avian sarcoma virus transforming gene product. Cell **15**:1363–1369.
11. **Coming, D. E.** 1973. A general theory of carcinogenesis. Proc. Natl. Acad. Sci. U.S.A. **70**:3324–3328.
12. **Curran, T., G. Peters, C. Van Beveren, N. M. Teich, and I. M. Verma.** 1982. FBJ murine osteosarcoma virus: identification and molecular cloning of biologically active proviral DNA. J. Virol. **44**:674–682.
13. **DeFeo, D., M. A. Gonda, H. A. Young, E. H. Chang, D. R. Lowy, E. M. Scolnick, and R. W. Ellis.** 1981. Analysis of two divergent rat genomic clones homologous to the transforming gene of Harvey murine sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **78**:3328–3332.
14. **Donner, L., L. A. Fedele, C. F. Garon, S. J. Anderson, and C. J. Sherr.** 1982. McDonough feline sarcoma virus: characterization of the molecularly cloned provirus and its feline oncogene (v-*fms*). J. Virol. **41**:489–500.
15. **Dziadek, M., and E. Adamson.** 1978. Localization and synthesis of alphafetoprotein in post-implantation mouse embryos. J. Embryol. Exp. Morphol. **43**:289–313.
16. **Ellis, R. W., D. DeFeo, M. E. Furth, and E. M. Scolnick.** 1982. Mouse cells contain two distinct *ras* gene mRNA species that can be translated into a p21 *onc* protein. Mol. Cell. Biol. **2**:1339–1345.
17. **Ellis, R. W., D. DeFeo, J. M. Maryak, H. A. Young, T. Y. Shih, E. H. Chang, D. R. Lowy, and E. M. Scolnick.** 1980. Dual evolutionary origin for the rat genetic sequences of Harvey murine sarcoma virus. J. Virol. **36**:408–420.
18. **Ellis, R. W., D. DeFeo, T. Y. Shih, M. A. Gonda, H. A. Young, N. Tsuchida, D. R. Lowy, and E. M. Scolnick.** 1981. The p21 *src* genes of Harvey and Kirsten sarcoma viruses originate from divergent members of a family of normal vertebrate genes. Nature (London) **292**:506–511.
19. **Frankel, A. E., and P. J. Fischinger.** 1976. Nucleotide sequences in mouse DNA and RNA specific for Moloney sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **73**:3705–3709.
20. **Frankel, A. E., J. H. Gilbert, K. G. Porzig, E. M. Scolnick, and S. A. Aaronson.** 1979. Nature and distribution of feline sarcoma virus nucleotide sequence. J. Virol. **30**:821–827.
21. **Gelman, E. P., F. Wong-Staal, R. A. Kramer, and R. C. Gallo.** 1981. Molecular cloning and comparative analysis of the genomes of simian sarcoma virus and its associated helper virus. Proc. Natl. Acad. Sci. U.S.A. **78**:3373–3377.
22. **Goff, S. P., E. Gilboa, O. N. Witte, and D. Baltimore.** 1980. Structure of the Abelson murine leukemia virus genome and the homologous cellular gene: studies with cloned viral DNA. Cell **22**:777–785.
23. **Gonda, T. J., D. K. Sheiness, and J. M. Bishop.** 1982.

Transcripts from the cellular homologs of retroviral oncogenes: distribution among chicken tissues. Mol. Cell. Biol. **2**:617–624.

24. **Graf, T., and H. Beug.** 1978. Avian leukemia viruses: interaction with their target cells in vivo and in vitro. Biochim. Biophys. Acta **516**:269–299.
25. **Harvey, J. J.** 1964. An unidentified virus which causes the rapid production of tumours in mice. Nature (London) **204**:1104–1105.
26. **Jones, M., R. A. Bosselman, V. J. F. Houtin, A. Berub, H. Fan, and I. M. Verma.** 1980. Identification and molecular cloning of Moloney mouse sarcoma virus-specific sequences from uninfected mouse cells. Proc. Natl. Acad. Sci. U.S.A. **77**:2651–2655.
27. **Kirsten, W. H., and L. A. Mayer.** 1967. Morphologic responses to a murine erythroblastosis virus. J. Natl. Cancer Inst. **39**:311–335.
28. **Langbeheim, H., T. Y. Shih, and E. M. Scolnick.** 1980. Identification of a normal vertebrate cell protein related to the p21 src of Harvey murine sarcoma virus. Virology **106**:292–300.
29. **Mathey-Prevot, B., H. Hanafusa, and S. Kawai.** 1982. A cellular protein is immunologically crossreactive with and functionally homologous to the Fujinami sarcoma virus transforming protein. Cell **28**:897–906.
30. **McDonough, S. K., S. Larsen, R. S. Brodey, N. D. Stock, and W. D. Hardy, Jr.** 1971. A transmissible feline fibrosarcoma of viral origin. Cancer Res. **31**:953–956.
31. **Müller, R., D. J. Slamon, J. M. Tremblay, M. J. Cline, and I. M. Verma.** 1982. Differential expression of cellular oncogenes during pre- and postnatal development of the mouse. Nature (London) **299**:640–644.
32. **Oppermann, H., A. D. Levinson, H. E. Varmus, L. Levintow, and J. M. Bishop.** 1979. Uninfected vertebrate cells contain a protein that is closely related to the product of the avian sarcoma virus transforming gene (src). Proc. Natl. Acad. Sci. U.S.A. **76**:1804–1808.
33. **Oskarsson, M., W. McClements, D. G. Blair, J. V. Maizel, and G. F. Vande Woude.** 1980. Properties of a normal mouse cell DNA sequence (*sarc*) homologous to the *src* sequence of Moloney sarcoma virus. Science **207**:1222–1224.
34. **Parker, R. C., H. E. Varmus, and J. M. Bishop.** 1981. Cellular homolog (c-*src*) of the transforming gene of Rous sarcoma virus: isolation, mapping, and transcriptional analysis of c-*src* and flanking regions. Proc. Natl. Acad. Sci. U.S.A. **78**:5842–5846.
35. **Perbal, B., and M. A. Baluda.** 1982. Avian myeloblastosis virus transforming gene is related to unique chicken DNA regions separated by at least one intervening sequence. J. Virol. **41**:250–257.
36. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity in vitro by nick-translation with DNA polymerase I. J. Mol. Biol. **113**:237–257.
37. **Roussel, M., S. Saule, C. Lagrou, C. Rommens, H. Beug, T. Graf, and D. Stehelin.** 1979. Three new types of viral oncogene of cellular origin specific for haematopoietic cell transformation. Nature (London) **281**:452–455.
38. **Scolnick, E. M., M. O. Weeks, T. Y. Shih, S. K. Ruscetti, and T. M. Dexter.** 1981. Markedly elevated levels of an endogenous *sarc* protein in a hematopoietic precursor cell line. Mol. Cell. Biol. **1**:66–74.
39. **Sheiness, D., and J. M. Bishop.** 1979. DNA and RNA from uninfected vertebrate cells contain nucleotide sequences related to the putative transforming gene of avian myelocytomatosis virus. J. Virol. **31**:514–521.
40. **Shibuya, M., H. Hanafusa, and P. C. Balduzzi.** 1982. Cellular sequences related to three new *onc* genes of avian sarcoma virus (*fps*, *yes*, and *ros*) and their expression in normal and transformed cells. J. Virol. **42**:143–152.
41. **Shilo, B. Z., and R. A. Weinberg.** 1981. DNA sequences homologous to vertebrate oncogenes are conserved in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S.A. **78**:6789–6792.
42. **Souza, L. M., J. N. Strommer, R. L. Hillyard, M. C. Komaromy, and M. A. Baluda.** 1980. Cellular sequences are present in the presumptive avian myeloblastosis virus genome. Proc. Natl. Acad. Sci. U.S.A. **77**:5177–5181.
43. **Spector, D. H., K. Smith, T. Padgett, P. McCombe, D. Roulland-Dussoix, C. Moscovici, H. E. Varmus, and J. M. Bishop.** 1978. Uninfected avian cells contain RNA related to the transforming gene of avian sarcoma viruses. Cell **13**:371–379.
44. **Spector, D. H., H. E. Varmus, and J. M. Bishop.** 1978. Nucleotide sequences related to the transforming gene(s) of avian sarcoma viruses are present in DNA of uninfected vertebrates. Proc. Natl. Acad. Sci. U.S.A. **75**:4102–4106.
45. **Stehelin, D., H. E. Varmus, J. M. Bishop, and P. K. Vogt.** 1976. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. Nature (London) **260**:170–173.
46. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. U.S.A. **77**:5201–5205.
47. **Tilghman, S. M., D. Kioussis, M. B. Gorin, J. P. Garcia Ruiz, and R. S. Ingram.** 1979. The presence of intervening sequences in the α-fetoprotein gene of the mouse. J. Biol. Chem. **254**:7393–7399.
48. **Vennström, B., and J. M. Bishop.** 1982. Isolation and characterization of chicken DNA homologous to the two putative oncogenes of avian erythroblastosis virus. Cell **28**:135–143.
49. **Wang, S. Y., W. S. Hayward, and H. Hanafusa.** 1977. Genetic variation in the RNA transcripts of endogenous virus genes in uninfected chicken cells. J. Virol. **24**:64–73.
50. **Weiss, R. A., N. M. Teich, H. M. Varmus, and J. Coffin (ed.).** 1982. Molecular biology of tumor viruses, 2nd ed., RNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
51. **Witte, O. N., N. Rosenberg, and D. Baltimore.** 1979. A normal cell protein cross-reactive to the major Abelson murine leukemia virus gene product. Nature (London) **281**:396–398.
52. **Wong-Staal, F., R. Dalla-Favera, G. Franchini, E. P. Gelman, and R. C. Gallo.** 1981. Three distinct genes in human DNA related to the transforming genes of mammalian sarcoma viruses. Science **213**:226–228.
53. **Yoshida, M., S. Kawai, and K. Toyoshima.** 1980. Uninfected avian cells contain structurally unrelated progenitors of viral sarcoma genes. Nature (London) **287**:653–654.