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Regulated Transcription of c-Ki-*ras* and c-*myc* During Compensatory Growth of Rat Liver

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We examined the transcription of six cellular oncogenes during the process of compensatory growth in rat liver after partial hepatectomy. We have previously reported that transcripts of c-*ras*^{H} are elevated during regenerative growth of the liver. We now report that transcripts of c-*ras*^{K} and c-*myc* genes are significantly elevated after partial hepatectomy, whereas transcripts of c-*abl* and c-*src* are essentially unchanged and transcripts of c-*mos* are undetectable in either normal or regenerating rat liver. In liver regeneration after partial hepatectomy or chemical injury, changes in c-*myc* transcripts occur before DNA synthesis. The elevation of c-*myc* and c-*ras* transcripts is sequential in that highest levels of c-*myc* transcripts were detected 12 to 18 h after partial hepatectomy, whereas the levels of c-*ras*^{H} and c-*ras*^{K} were maximal by 36 to 48 h. Transcripts of all three activated oncogenes returned to their basal levels by 96 h.

The study of RNA tumor viruses has defined a set of genes (oncogenes) capable of inducing neoplastic transformation. Subsequent analysis has revealed that viral oncogenes (v-*onc*) are very closely related to sequences present in the genome of normal cells (c-*onc*). Retroviruses acquire oncogenic sequences from cells in a process analogous to the incorporation of genetic material from host cells by transducing bacteriophages. At present, more than 20 oncogenes, identified by their association with RNA tumor viruses or by DNA-mediated gene transfer (transfection), have been described (2–4, 8).

We know a great deal about the role of some of the viral oncogenes in cell transformation but almost nothing about the function of their normal cell counterparts. Given that these genes are highly conserved during evolution (2, 3), there is reason to believe that cellular oncogenes play some essential role in the control of normal growth, embryogenesis, or differentiation. Experimental evidence for the regulated expression of cellular oncogenes in nonneoplastic growth is, however, meager.

Regeneration of rat liver after partial hepatectomy or chemical injury is a good experimental system to study the reentry of normally quiescent cells into the cell cycle (6). The sequence of events which takes place during compensatory growth of rat liver after partial hepatectomy has been extensively described, but many of the mechanisms controlling the different stages of the process remain to be uncovered. During the prereplicative stage (hypertrophy), there are increases in ribosomal RNA, in the size of polysomes, and in the amount of polyadenylated [poly(A)$^+$] mRNA in polysomes (1, 16). Hepatocyte DNA replication (hyperplasia) starts ca. 12 to 24 h after partial hepatectomy, reaches a maximum at 24 h, and is followed 6 to 8 h later by a wave of mitosis (23). A second peak of DNA synthesis is detectable at 40 to 48 h after the operation (6, 23). During the regeneration of rat liver caused by CCl_4 injury, maximal levels of DNA synthesis occur 48 h after CCl_4 administration. Other peaks of DNA synthesis are not readily identifiable after CCl_4 injury, apparently because of the loss of synchrony in cell division after the first wave.

Polysomal poly(A)$^+$ mRNA populations in normal and regenerating liver appear to be very similar, if not identical. However, there are obvious changes in the abundance of some sequences after partial hepatectomy (16, 17, 22, 41, 46). We have reported that transcripts of the c-*ras*^{H} gene increase during liver regeneration after partial hepatectomy or CCl_4 injury in rats and that this increase roughly parallels the first wave of DNA synthesis during regenerative growth (18, 21). We now present data on the RNA levels of six cellular oncogenes (*ras*^{H}, *ras*^{K}, *myc*, *mos*, *abl*, and *src*) in polysomal poly(A)$^+$ RNA populations obtained at various times after partial hepatectomy and on the sizes of transcripts of the c-*ras*^{H}, c-*ras*^{K}, and c-*myc* genes. mRNAs corresponding to c-*ras*^{H}, c-*ras*^{K}, and c-*myc* increase markedly during liver regeneration, without alterations in the size of the transcripts. The kinetics of the elevation of these sequences suggest that during compensatory growth of the liver, c-*myc* activation precedes that of c-*ras*^{K} or c-*ras*^{H}. In contrast, the abundance of c-*src* and c-*abl* transcripts does not change during liver regeneration. Transcripts from the *mos* gene were not detected in normal or regenerating livers.

MATERIALS AND METHODS

Animals. Male albino rats (Holtzman strain, Crl: CDHSDBR, Charles River Laboratory) weighing 120 to 140 g were used for all experiments. Partial hepatectomies were performed by the method of Higgins and Anderson (25). Conditions pertaining to animal maintenance and surgery have been described previously (17, 41). In experiments involving CCl_4 injury, rats received a single intragastric dose of 0.5 ml of 50% CCl_4 (diluted in olive oil) per 100 g of body weight (37).

RNA extraction. Livers were homogenized, and polysomes were isolated by magnesium precipitation as previously described (17, 37, 41). Polysomal RNA was extracted with phenol-chloroform and fractionated by polyuridylate-Sepharose affinity chromatography to obtain poly(A)$^+$ mRNA.

Determination of size of oncogene transcripts. Liver polysomal mRNA was separated on 1.1 or 1.5% agarose-formaldehyde gels and transferred to nitrocellulose paper (5). ^{32}P-end-labeled *Hin*dIII fragments of λ DNA were used as size markers.

Hybridization and DNA probes. ^{32}P-labeled probes were

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prepared by nick translation (39) from the following viral oncogene clones: the BS-9 insert for ras^H (13); the HiHi 380 fragment for ras^K (13); the 2.2-kilobase (kb) *Pst*I fragment of v-*myc* for *myc* (45); pABLsub9 for *abl* (J. Y. W. Wang and D. Baltimore, personal communication); the pHT10 insert of v-*mos* for *mos* (35); the 3-kb *Eco*RI fragment of v-*src* for *src* (9). The method of Thomas (44) was used for dot-blot hybridizations (21). Liver mRNA (1 and 5 μg) in 5 μl of water was spotted onto nitrocellulose filters and incubated with labeled probes in hybridization buffer for 72 h at 42°C as described previously (21). After the hybridization, filters were washed, and autoradiographs were prepared. Subsequently, the filter spots were punched out and counted in Omnifluor-toluene. Standards ranging from 1.5 to 200 pg of each oncogene DNA were included on the corresponding filters to determine the sensitivity of the procedure. Background radioactivity was determined by punching out random portions of the filters not containing RNA spots. These values were subtracted from the radioactivity found in each RNA spot.

Determination of c-ras^K transcripts. Preliminary experiments indicated that the HiHi 380 probe hybridizes with BS-9 DNA under the hybridization conditions described above. However, when the temperature of hybridization was raised to 50°C, there was no cross hybridization between the HiHi 380 (ras^K) and BS-9 (ras^H) probes (data not shown). All hybridization reactions involving the HiHi 380 probe described in this paper were done at 50°C. Under these conditions, the ras^H and ras^K probes detected distinct RNA transcripts (see below).

RESULTS

To determine the sensitivity of the RNA blotting procedure, various amounts of liver polysomal poly(A)$^+$ RNA were electrophoresed in 1.1% agarose-formaldehyde gels, transferred to filters, and hybridized with labeled BS-9 DNA (ras^H). There was a linear increase in the intensity of the labeled band at 1.2 kb as the amount of RNA blotted was increased from 0.5 to 5 μg (Fig. 1A and B).

We compared the amount of c-ras^K and c-ras^H transcripts in polysomal poly(A)$^+$ RNA obtained from livers of normal and sham-operated rats and from regenerating livers at 12, 18, 24, 36, 48, and 96 h after partial hepatectomy by using the dot-blot procedure. Transcripts of c-ras^K increased dramatically during regenerative growth, reaching a maximum by 36 h and returning to basal levels at 96 h. At 24 h after partial hepatectomy, the levels of c-ras^K transcripts in polysomal poly(A)$^+$ RNA were at least 10 times higher than those in sham-operated rats and 20 to 50 times higher than the amounts detected in mRNA preparations from normal livers (Fig. 2). As previously described (18, 21), c-ras^H transcripts also increased during liver regeneration (Fig. 3a), and their elevation also roughly coincided with the first and major wave of DNA synthesis during liver regeneration after partial hepatectomy. Maximum levels of c-ras^H transcripts in regenerating liver were approximately three to five times higher than normal. Because of the relatively low levels of expression of these genes in normal and sham-operated rats, the exact magnitude of the increase occurring after partial hepatectomy was difficult to determine. However, the changes in hybridization detected with regenerating liver mRNA relative to mRNA from livers of normal or sham-operated rats proved to be reproducible with different RNA preparations (each obtained from 8 to 10 rats). The number of counts hybridized in each experiment varied with the specific activity of the labeled DNA probe.

Recent studies suggest that *myc* and *ras* genes interact in inducing transformation in primary cell cultures (27). We

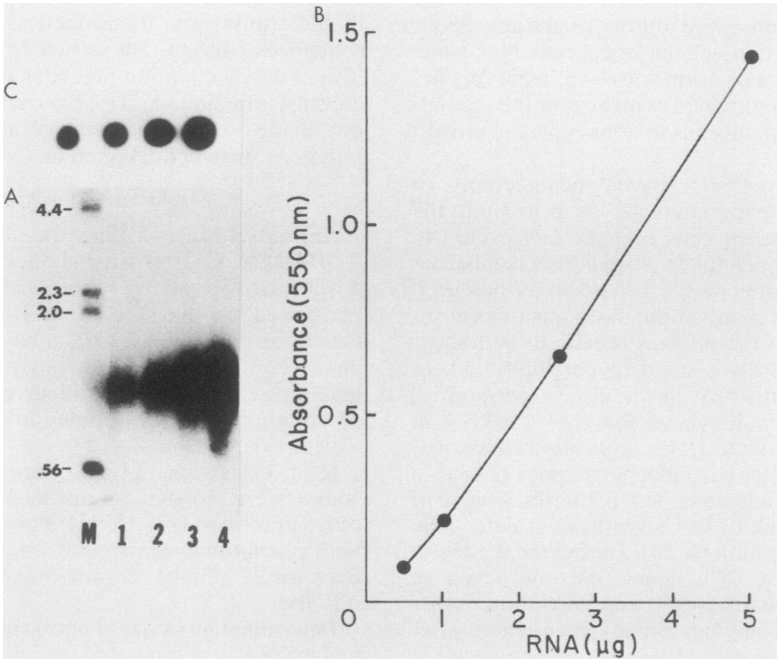

FIG. 1. Sensitivity of the RNA blotting procedure. (A) Various amounts of polysomal poly(A)$^+$ RNA from 36-h regenerating liver (posthepatectomy) were fractionated in agarose-formaldehyde gels and hybridized to ^{32}P-labeled v-ras^H DNA as described in the text. Lane M, *Hin*dIII digest of phage λ DNA; lanes 1 to 4, 0.5, 1.0, 2.5, and 5 μg of mRNA from 36-h regenerating liver. RNA was prepared from eight rats. (B) Spectrophotometric scanning of Northern blot shown in (A). (C) Dot-blot hybridization of various amounts of unlabeled BS-9 DNA to ^{32}P-labeled BS-9 DNA. The dots contain 12.5, 25, 50, and 100 pg of BS-9 DNA (left to right).

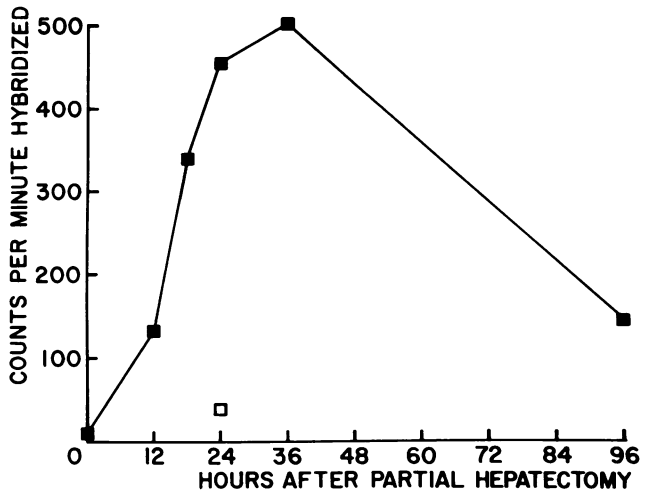

FIG. 2. c-*ras*^{K} transcripts during liver regeneration. Polysomal poly(A)$^+$ RNAs (5 μg) extracted from normal liver, from regenerating liver (■), and from the liver of 24-h sham-operated animals (□) were spotted onto nitrocellulose filters and hybridized to a ^{32}P-labeled v-*ras*^{K} probe as described in the text. The radioactivity of each of the RNA spots was determined. The values given on the ordinate are the counts per minute in the spots minus the background value. The abscissa shows the time (after partial hepatectomy) at which the animals were killed. Each RNA preparation was obtained from livers of 8 to 10 rats.

therefore analyzed the expression of the c-*myc* gene during liver regeneration and compared its pattern of change with that of c-*ras*^{H,K}. These studies were done in two different systems in which regeneration of the liver occurs, partial hepatectomy and chemical injury with CCl_4.

In livers damaged by CCl_4, an increase in DNA synthesis was not detected until 24 h after the administration of the chemical and reached a maximum at 48 h. Thus, the major wave of DNA synthesis in this experimental system occurred 24 h later than in the regeneration of the liver after partial hepatectomy. In both regenerative processes (Fig. 3A and B), there was a considerable increase in c-*myc* transcripts (fivefold at 18 h after partial hepatectomy and 24 h after CCl_4 injury). After partial hepatectomy, c-*myc* transcripts reached maximum levels ca. 12 to 18 h before the maximal increase in c-*ras* gene expression and 6 h before the time of maximal DNA synthesis (Fig. 3A). After CCl_4 injury, maximal elevation of c-*myc* transcripts preceded the maximal changes in c-*ras*^{H} transcripts and the peak of DNA synthesis by about 24 h (Fig. 3B). A second but smaller elevation in c-*myc* transcripts occurred 48 h after partial hepatectomy, coinciding with the second, less prominent wave of DNA replication (Fig. 3A).

To determine whether the elevation of c-*ras*^{H}, c-*ras*^{K}, and c-*myc* transcripts are part of a general activation of cellular oncogenes during regenerative growth, we hybridized probes representing the oncogenes of Abelson murine leukemia virus (v-*abl*), Moloney murine sarcoma virus (v-*mos*), and Rous sarcoma virus (v-*src*) with liver polysomal poly(A)$^+$ RNA. Transcripts from c-*mos* were not detected in RNA from normal or regenerating liver; c-*abl* transcripts were barely detectable in our preparation from normal or regenerating liver; c-*src* transcripts were easily detected in both normal and regenerating liver, but no changes were observed. Thus, we found no elevation of the transcripts of these three oncogenes during liver regeneration (data not shown).

We determined whether there are changes in the size of c-*ras*^{H}, c-*ras*^{K}, and c-*myc* transcripts (Fig. 4) during liver regeneration. Five-microgram samples of the various mRNAs were separated by electrophoresis in 1.1 or 1.5% agarose-formaldehyde gels and transferred to nitrocellulose filters as described above. For normal and regenerative liver, Northern blots revealed a 1.2-kb transcript for the c-*ras*^{H} gene and two transcripts of the c-*ras*^{K} gene corresponding to 2.1 and 5.0 kb. The sizes of these transcripts are similar to those described in mouse tissues (12, 31). Multiple transcripts of the c-*myc* gene are detectable by the avian v-*myc* probe. The most obvious bands migrate at 2.6, 2.0, and 1.8 kb and are present in both normal and regenerative liver. Multiple c-*myc* transcripts have also been detected in some mouse plasmacytomas (33). The data in Fig. 4 confirm the elevation of c-*ras*^{K}, c-*ras*^{H}, and c-*myc* transcripts during liver regeneration, demonstrated with the dot-blot procedure.

We estimated the numbers of copies per cell of c-*ras*^{H} transcripts in normal and regenerating liver by using the data shown in Fig. 1. To relate the signals generated by known amounts of poly(A)$^+$ RNA to that of the BS-9 probe, the films from the Northern gels (Fig. 1A) and dot-blot hybridization between the BS-9 probe and unlabeled BS-9 DNA (Fig. 1C) were scanned in a spectrophotometer to generate standard curves. To make direct comparisons between DNA/DNA and DNA/RNA hybridizations, adjustments were made to compensate for the difference in size between the BS-9 probe (460 base pairs) and the c-*ras*^{H} transcripts (1.2 kb). An additional correction is applied because both strands of the DNA probe contribute to the hybridization signal. Polysomal poly(A)$^+$ RNA from 36-h regenerating liver contained ca. 50 to 75 pg of c-*ras*^{H} mRNA per 5 μg or roughly 0.001%. If c-*ras*^{H} transcripts were equally distributed in all liver cells, their abundance would be in the range of 5 to 10 copies per cell, a value similar to that calculated for hepatic α-fetoprotein mRNA (37). If only replicating hepatocytes have an elevated number of c-*ras*^{H} transcripts, these sequences could have an abundance of 20 to 40 copies per cell (hepatocytes constitute ca. 60 to 65% of all liver cells, and the assumption is made that 24 h after partial hepatectomy, about 40% are in S phase). Corresponding values for c-*ras*^{K} transcripts would be in the range of 100 to 200 copies per cell. These values represent only approximations and are highly dependent on the distribution of transcripts in various cell types in rat liver.

DISCUSSION

The role of oncogenes in viral transformation has been firmly established by both genetic and biochemical studies. We now know the cellular location of a number of oncogenic proteins and, in some cases, the enzymatic and physical properties of the proteins (2–4, 8). However, the role of the cellular oncogenes in normal cellular processes is at present, poorly defined. There have been two basic experimental designs to study the role of cellular oncogenes in normal tissues in vivo. The first involves the examination of various tissues of an animal for differential expression of these genes; the other consists of analyses of cellular oncogene expression in embryos at different stages of development and neonatal animals (19, 31, 32). Although some specificity of oncogene transcription in different tissues, stages of embryonic development, and extraembryonal membranes has been described, it is not yet possible to draw general conclusions from the data. Obviously, these studies must be coupled with the establishment of an appropriate in vitro system which will permit the study of the role of oncogenes

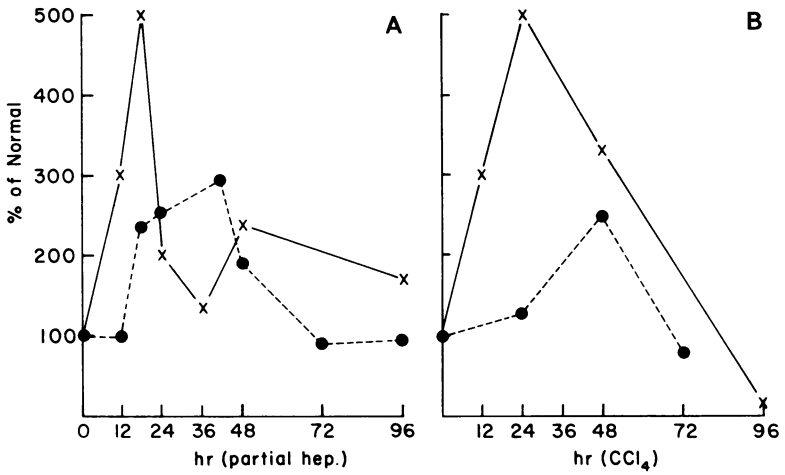

FIG. 3. c-*myc* and c-*ras*^{H} transcripts during liver regeneration after partial hepatectomy (A) or CCl_4 administration (B). Polysomal poly(A)$^+$ RNAs (5 μg) extracted from normal liver and from regenerating livers were spotted onto nitrocellulose filters and hybridized to a ^{32}P-labeled v-*ras*^{H} probe (●) and to a ^{32}P-labeled v-*myc* probe (×) as described in the text. The hybridization values shown on the ordinate are expressed as percentage of normal, relative to values obtained by hybridization of liver RNA from normal rats to the same probes (150 cpm for the *ras* probe and 60 cpm for the *myc* probe, after subtraction of background values). The abscissa shows the time after partial hepatectomy or CCl_4 administration at which the animals were killed. Each RNA preparation was obtained from 8 to 10 partially hepatectomized rats and 3 to 5 rats which received CCl_4 intragastrically. Curves for c-*ras*^{H} transcripts were redrawn from reference 21 (dashed lines).

in the normal cell cycle and in the neoplastic transformation of these cells.

Regenerative growth of the liver provides an interesting system to examine the role of cellular oncogenes in nonneoplastic growth. After partial hepatectomy, cells reenter the cell cycle in a reasonably synchronous manner leading to a major wave of DNA replication at 24 h, followed by a mitotic wave 6 to 8 h later. In young animals, a second peak of DNA synthesis is detectable at 48 h, reflecting DNA replication in ductal and littoral cells and in hepatocytes going through a second round of cell division (6, 23). Although a low level of DNA synthesis persists in the organ, distinct peaks of DNA synthesis are no longer detectable 96 h after the operation. Thus, in this system, it is possible to measure cellular oncogene expression in mitotically quiescent cells, during cellular hypertrophy and hyperplasia, and when cells return to a resting state.

Although there is an increase in cytoplasmic poly(A)$^+$

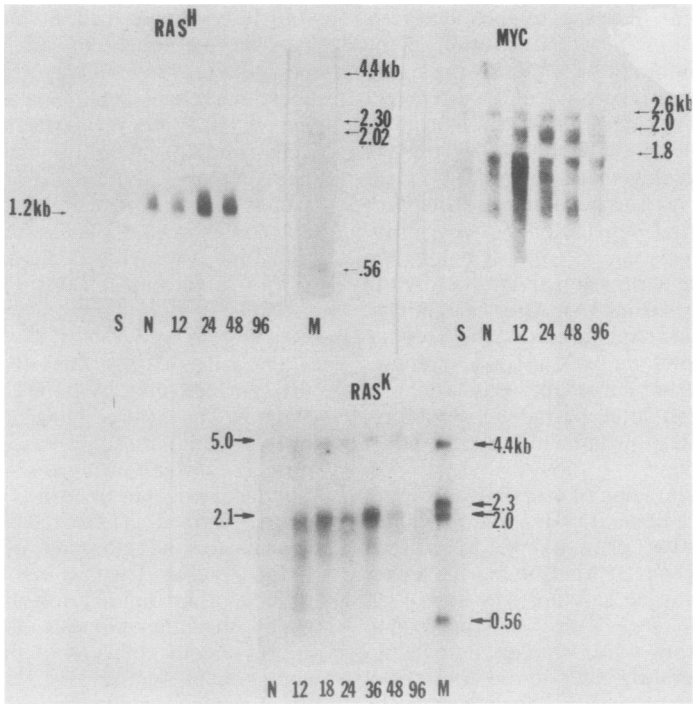

FIG. 4. Analysis of c-*myc*, c-*ras*^{H}, and c-*ras*^{K} mRNAs during liver regeneration. Polysomal poly(A)$^+$ RNA (5 μg) from normal liver (N), from liver 24 h after sham operation (S), and from regenerating liver at various times after partial hepatectomy were fractionated in agarose-formaldehyde gels, blotted to nitrocellulose, and hybridized to the appropriate ^{32}P-labeled probe as described in the text. The marker lane (M) contains a *Hin*dIII digest of phage λ DNA. The filter used for hybridization to the v-*myc* probe was later washed and used in the hybridization to the v-*ras*^{H} probe.

mRNA in the first 6 to 12 h after partial hepatectomy (1), measurements of the percentage of single-copy DNA transcribed in normal and regenerating mRNA populations reveal essentially no differences. Furthermore, analysis of the complexity and homology between poly(A)$^+$ mRNA populations from normal and regenerating liver do not show statistically significant differences. These and other experiments suggest that the increase in polysomal poly(A)$^+$ mRNA at the early stages of liver regeneration must represent the transcription of mRNAs which are already present in normal livers, although none of the available data preclude minor qualitative changes (16, 17, 22, 41, 46). Despite the close qualitative homology between mRNA populations in normal and regenerating liver, there are differences in the abundance of certain transcripts between these populations. Our results with cellular oncogenes are in accord with these observations.

We detected a 2- to 10-fold increase in transcripts of c-*ras*^{H}, c-*ras*^{K}, and c-*myc*. The elevation is highest for the c-*ras*^{K} gene. The magnitude of the increase in cellular gene transcripts is significantly greater than the overall increase in polysomal poly(A)$^+$ mRNA or the changes in α-fetoprotein and albumin mRNA after partial hepatectomy (18, 37). Regeneration does not result in general activation of cellular oncogenes since c-*abl* and c-*src* transcripts remain essentially unchanged and c-*mos* transcripts are not detected.

The c-*ras*^{K} oncogene is activated in human colon, lung, and pancreatic carcinomas (38), in human cell lines derived from lung, colon, and bladder tumors (10, 30, 43), in mouse adrenal tumors (42), and in fibroblasts transformed by 3-methyl-cholanthrene (14, 36). In addition to its activation in human, murine, and avian hemopoietic neoplasias (24, 29, 33), c-*myc* is amplified in human lung cancer cell lines (28) and highly expressed in cell lines derived from human lung and ovarian tumors (15). It is intriguing that transcripts of both c-*myc* and c-*ras*^{H} are elevated during regenerative growth in what appears to be a sequential manner. It has been known for some time that c-*myc* acts in concert with a second oncogene, c-*blym*, in virus-induced bursal lymphomas of chickens (20). The same two oncogenes appear to be implicated in the genesis of Burkitt lymphomas as well (11). Recently, Land et al. (27) reported that *myc* can function in conjunction with activated c-*ras*^{H} genes isolated from the EJ human bladder carcinoma cell line to transform normal rat fibroblasts in culture. These and other results (34, 40) suggest that oncogenes may cooperate in a multistep process of transformation. Oncogene products which are located in the nucleus, such as *myc*, adenovirus E1A, or polyoma large T-antigen, may function to immortalize cells, whereas those which reside in the plasma membrane, such as *ras* or polyoma middle-T antigen, may transform cells if they have been immortalized (27, 40). Clearly, there may be more or different steps in the process of transformation, but these results may be quite relevant for understanding the triggering of liver regeneration. Since enhanced transcription of c-*myc* precedes DNA synthesis by 12 h, it is conceivable that this gene plays a role in triggering the S phase in hepatocytes or acts at an even earlier stage, in inducing hepatocytes to enter the cell cycle. In support of such view is the observation that when cells arrested in G1 are stimulated to divide, transcription of c-*myc* is dramatically elevated (7, 26). The kinetics of induction of c-*ras*^{H} and c-*ras*^{K} suggest a role concomitant with or subsequent to DNA synthesis, perhaps in mitosis. The activation of c-*myc* during the early stages of liver regeneration may alter the response of hepatocytes to humoral factors or may in itself be a result of the action of one or more of these factors or of changes in the composition of plasma brought about by the functional demands on the liver after partial hepatectomy.

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LITERATURE CITED

1. **Atryzek, V., and N. Fausto.** 1979. Accumulation of polyadenylated mRNA during liver regeneration. Biochemistry **18**:1281–1287.
2. **Bishop, J. M.** 1983. Cellular oncogenes and retroviruses. Annu. Rev. Biochem. **52**:301–354.
3. **Bishop, J. M., and H. E. Varmus.** 1982. Functions and origins of retroviral tranforming genes, p. 999–1108. *In* R. A. Weis, N. Teich, H. Varmus, and J. Coffin (ed.), Molecular biology of RNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
4. **Bister, K., and P. H. Duesberg.** 1982. Genetic structure and transforming genes of avian retroviruses, p. 3–42. *In* G. Klein (ed.), Advances in viral oncology, vol. 1. Raven Press, New York.
5. **Bostian, K. A., J. M. Lemire, and H. O. Halvorson.** 1983. Physiological control of repressible acid phosphatase gene transcripts in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **3**:839–853.
6. **Bucher, N. L. R., and R. A. Malt.** 1971. Regeneration of liver and kidney. Little, Brown & Co., Boston.
7. **Campisi, J., H. E. Gray, A. B. Pardee, M. Dean, and G. E. Sonenshein.** 1984. Cell cycle control of c-*myc* but not c-*ras* expression is lost following chemical transformation. Cell **36**:241–247.
8. **Cooper, G. M.** 1982. Cellular transforming genes. Science **218**:801–806.
9. **DeLorbe, W. J., P. A. Luciw, H. M. Goodman, H. E. Varmus, and J. M. Bishop.** 1980. Molecular cloning and characterization of avian sarcoma virus circular DNA molecules. J. Virol. **36**:50–61.
10. **Der, C., T. Krontiris, and G. M. Cooper.** 1982. Transforming genes of human bladder and lung carcinoma lines are homologous to the *ras* genes of Harvey and Kirsten sarcoma viruses. Proc. Natl. Acad. Sci. U.S.A. **79**:3637–3640.
11. **Diamond, A., G. M. Cooper, J. Ritz, and M. A. Lane.** 1983. Identification and molecular cloning of the human *Blym* transforming gene activated in Burkitt's lymphomas. Nature (London) **305**:112–116.
12. **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.
13. **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**:506–508.
14. **Eva, A., and S. A. Aaronson.** 1983. Frequent activation of c-Ki-*ras* as a transforming gene in fibrosarcomas induced by methylcholanthrene. Science **220**:955–956.
15. **Eva, A., K. C. Robbins, P. R. Andersen, A. Srinivasan, S. R. Tsonick, E. P. Reddy, N. W. Ellmore, A. T. Galen, J. A. Lautenberger, T. S. Papas, E. H. Westin, F. Wong-Staal, R. C. Gallo, and S. A. Aaronson.** 1982. Cellular genes analogous to retroviral *onc* genes are transcribed in human tumour cells. Nature (London) **295**:116–119.
16. **Fausto, N.** 1984. Messenger RNA in regenerating liver: implications for the understanding of regulated growth. Mol. Cell. Biochem. **59**:131–147.

17. **Fausto, N., G. Schultz-Ellison, V. Atryzek, and M. Goyette.** 1982. Distribution and specificity of sequences in polyadenylated nuclear RNA of normal, regenerating and neoplastic liver. J. Biol. Chem. **257**:2200–2206.
18. **Fausto, N., and P. R. Shank.** 1983. Oncogene expression in liver regeneration and hepatocarcinogenesis. Hepatology **3**:1016–1023.
19. **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.
20. **Goubin, G., D. S. Goldman, J. Luce, P. E. Neiman, and G. M. Cooper.** 1983. Molecular cloning and nucleotide sequence of a transforming gene detected by transfection of chicken B-cell lymphoma DNA. Nature (London) **302**:114–119.
21. **Goyette, M., C. J. Petropoulos, P. R. Shank, and N. Fausto.** 1983. Expression of a cellular oncogene during liver regeneration. Science **219**:510–512.
22. **Grady, L. J., W. P. Campbell, and A. B. North.** 1981. Sequence diversity of nuclear and polysomal polyadenylated and non-polyadenylated RNA in normal and regenerating rat liver. Eur. J. Biochem. **115**:241–245.
23. **Grisham, J. W.** 1962. Morphologic study of deoxyribonucleic acid synthesis and cell proliferation in regenerating rat liver: autoradiography with thymidine-H^3. Cancer Res. **22**:842–849.
24. **Hayward, W. S., B. G. Neel, and S. M. Astrin.** 1982. Avian leukosis viruses: activation of cellular oncogenes, p. 207–233. *In* G. Klein (ed.), Advances in viral oncology, vol. 1. Raven Press, New York.
25. **Higgins, G. M., and R. M. Anderson.** 1931. Experimental pathology of the liver. I. Restoration of the liver of the white rat following partial surgical removal. Arch. Pathol. **12**:186–202.
26. **Kelly, K., B. H. Cochran, C. D. Stiles, and P. Leder.** 1983. Cell-specific regulation of the c-*myc* gene by lymphocyte mitogens and platelet-derived growth factor. Cell **35**:603–610.
27. **Land, H., L. F. Parada, and R. A. Weinberg.** 1983. Tumorgenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature (London) **304**:596–602.
28. **Little, C. D., M. M. Nau, D. N. Carney, A. F. Gazdar, and J. D. Minna.** 1983. Amplification and expression of the c-*myc* oncogene in human lung cancer cell lines. Nature (London) **306**:194–196.
29. **Maguire, R. T., T. S. Robins, S. S. Thorgeirsson, and C. A. Heilman.** 1983. Expression of cellular *myc* and *mos* genes in undifferentiated B cell lymphomas of Burkitt and non-Burkitt types. Proc. Natl. Acad. Sci. U.S.A. **80**:1947–1950.
30. **McCoy, M. S., J. J. Toole, J. M. Cunningham, E. H. Chang, D. R. Lowy, and R. A. Weinberg.** 1983. Characterization of a human colon/lung carcinoma oncogene. Nature (London) **302**:79–81.
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. **Müller, R., I. M. Verma, and E. D. Adamson.** 1983. Expression of c-*onc* genes: c-*fos* transcripts accumulate to high levels during development of mouse placenta, yolk sac and amnion. EMBO J. **2**:679–684.
33. **Mushinski, J. F., S. R. Bauer, M. Potter, and E. P. Reddy.** 1983. Increased expression of *myc*-related oncogene mRNA characterizes most BALB/c plasmacytomas induced by pristane or Abelson murine leukemia virus. Proc. Natl. Acad. Sci. U.S.A. **80**:1073–1077.
34. **Newbold, R. F., and R. W. Overell.** 1983. Fibroblast immortality is a prerequisite for transformation by EJ c-Ha-ras oncogene. Nature (London) **304**:648–651.
35. **Oskarsson, M., W. L. 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.
36. **Parada, L. F., and R. A. Weinberg.** 1983. Presence of a Kirsten murine sarcoma virus *ras* oncogene in cells transformed by 3-methylcholanthrene. Mol. Cell. Biol. **3**:2298–2301.
37. **Petropoulos, C., G. Andrews, T. Tamaoki, and N. Fausto.** 1983. α-Fetoprotein and albumin mRNA levels in liver regeneration and carcinogenesis. J. Biol. Chem. **258**:4901–4906.
38. **Pulciani, S., E. Santos, A. V. Lauver, L. K. Long, S. A. Aaronson, and M. Barbacid.** 1982. Oncogenes in solid human tumors. Nature (London) **300**:539–542.
39. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
40. **Ruley, H. E.** 1983. Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture. Nature (London) **304**:602–606.
41. **Scholla, C. A., M. V. Tedeschi, and N. Fausto.** 1980. Gene expression and the diversity of polysomal messenger RNA sequences in regenerating liver. J. Biol. Chem. **255**:2855–2860.
42. **Schwab, M., K. Alitalo, H. E. Varmus, J. M. Bishop, and D. George.** 1983. A cellular oncogene (c-Ki-*ras*) is amplified, overexpressed and located within karyotypic abnormalities in mouse adrenocortical tumor cells. Nature (London) **303**:497–501.
43. **Taparowsky, E., Y. Suard, O. Fasano, K. Shimizu, M. Goldfarb, and M. Wigler.** 1982. Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change. Nature (London) **300**:762–765.
44. **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.
45. **Venstrom, B., D. Sheiness, J. Zabielski, and J. M. Bishop.** 1982. Isolation and characterization of c-*myc*, a cellular homolog of the oncogene (v-*myc*) of avian myelocytomatosis virus strain 29. J. Virol. **42**:773–779.
46. **Wilkes, P. R., G. D. Birnie, and J. Paul.** 1979. Changes in nuclear and polysomal polyadenylated RNA sequences during rat liver regeneration. Nucleic Acids Res. **6**:2193–2208.