MOLECULAR AND CELLULAR BIOLOGY, June 1990, p. 3185–3193
0270-7306/90/063185-09$02.00/0

Vol. 10, No. 6

c-*myc*, c-*fos*, and c-*jun* Regulation in the Regenerating Livers of Normal and H-2K/c-*myc* Transgenic Mice

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Received 14 November 1989/Accepted 6 March 1990

We investigated the mechanisms of regulation of c-*myc*, c-*fos*, and c-*jun* at the early stages of liver regeneration in mice. We show that the transient increase in steady-state levels of c-*myc* mRNA at the start of liver regeneration is most probably regulated by posttranscriptional mechanisms. Although there was a marked increase in c-*myc* transcriptional initiation shortly after partial hepatectomy, a block in elongation prevented the completion of most transcripts. To gain further information on the mechanism of regulation of c-*myc* expression during liver regeneration, we used transgenic mice harboring the human c-*myc* gene driven by the H-2K promoter. In these animals, the murine c-*myc* responded to the growth stimulus generated by partial hepatectomy, whereas the expression of the transgene was constitutive and did not change in the regenerating liver. However, the mRNA from both genes increased markedly after cycloheximide injection, suggesting that the regulation of c-*myc* mRNA abundance in the regenerating liver differs from that occurring after protein synthesis inhibition. Furthermore, we show that in normal mice c-*fos* and c-*jun* mRNA levels and transcriptional rates increase within 30 min after partial hepatectomy. c-*fos* transcriptional elongation was restricted in nongrowing liver, but the block was partially relieved in the regenerating liver. Nevertheless, for both c-*fos* and c-*jun*, changes in steady-state mRNA detected after partial hepatectomy were much greater than the transcriptional increase. In the regenerating liver of H-2K/c-*myc* mice, c-*fos* and c-*jun* expression was diminished, whereas mouse c-*myc* expression was enhanced in comparison with that in nontransgenic animals.

Although the regulation of the expression of the proto-oncogenes c-*myc*, c-*fos*, and c-*jun* in cells in culture stimulated to grow by serum or specific mitogens has been investigated in detail, little is known about the mechanisms that control these genes during tissue growth in vivo (8, 23, 29, 37, 46, 48, 53). Liver regeneration after partial hepatectomy is a synchronized growth process during which normally quiescent hepatocytes undergo one or more rounds of replication to restore organ mass. In young rats and mice the growth response involves the replication of more than 95% of hepatocytes in the first 2 days after the operation. Because this rapid growth response takes place in intact liver lobes in the absence of wound or inflammation, the system has been widely used for studies of growth regulation in vivo (19).

During liver regeneration in rats there is a sequential and regulated expression of several proto-oncogenes (12, 13, 20, 22, 26, 32, 36, 58). In the first 2 to 4 h after partial hepatectomy there are transient changes in the steady-state levels of c-*fos*, c-*jun* (J. E. Mead, M. FitzGerald, and N. Fausto, unpublished data), and c-*myc* mRNAs as quiescent hepatocytes enter the cell cycle. c-*fos* and c-*jun* transcript levels increase in the liver almost immediately after partial hepatectomy and return to normal by 2 h. This is followed by an elevation of the c-*myc* mRNA level, which is highest at 2 h but decreases to the basal level at 4 h. On the basis of this pattern of proto-oncogene expression, we have divided the prereplicative phase of liver regeneration into priming and progression stages and have identified growth factors involved in the positive and negative control of rat liver growth in vivo (19, 20, 41). However, no information is available about the mechanisms responsible for the transient changes in abundance of c-*myc*, c-*fos*, and c-*jun* transcripts in regenerating rat or mouse liver. This question is of particular importance as the proteins coded by these genes probably modulate the activity of other genes necessary for cell cycle progression (9, 50, 51, 53) and replication of hepatocytes.

Here we present data on the regulation of c-*fos*, c-*jun*, and c-*myc* expression at the early stages of liver regeneration in mice, with emphasis on c-*myc*. c-*myc* regulation is complex and can take place at the transcriptional and posttranscriptional levels. Transcriptional regulation can occur at both the initiation and completion steps (5, 6, 18, 28, 35, 39, 48, 49), whereas posttranscriptional control may involve a change in transcript stability (8, 15–17), intranuclear processing, and nucleocytoplasmic transport. Furthermore, transcription in the antisense direction may contribute to the regulation of c-*myc* expression (47). For cells in culture the importance of each of these mechanisms varies depending on the cell system and the nature of the inducing agent (46). Recent data from normal and H-2K/c-*myc* transgenic mice indicate that c-*myc* regulation in various intact tissues including liver depends mostly on posttranscriptional mechanisms (42). We asked whether the expression of c-*myc*, c-*fos*, and c-*jun* would be regulated at the transcriptional or posttranscriptional levels during liver regeneration in mice and investigated whether the increase in steady-state c-*myc* and c-*fos* mRNA level is associated with modifications of the rates of transcriptional initiation and elongation.

To learn more about the nature of the mechanisms that may operate in vivo in the regulation of c-*myc* expression during liver regeneration, we used transgenic mice harboring the human c-*myc* gene driven by the class I H-2K promoter (42, 43). We investigated whether expression of the transgene, which has intact P2 promoter sequences in exon 1 and unmodified exons 2 and 3, would be modulated during liver growth. Furthermore, to find whether destabilizing elements

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might play a role in the transient increase in c-*myc* transcripts in the regenerating liver, we determined whether the amount of transgene and murine c-*myc* mRNAs are affected by protein synthesis inhibition caused by cycloheximide injection. Finally, we examined whether the timing and extent of c-*fos* and c-*jun* expression after partial hepatectomy would be altered in H-2K/c-*myc* transgenic mice.

MATERIALS AND METHODS

Animals. Two independent lines of H-2K/c-*myc* transgenic mice (strains 14 and 27 [43]) and their nontransgenic littermates (6 to 8 months old) were used in this study. All experiments were done with H-2K/c-*myc* strain 27, and some were repeated with H-2K/c-*myc* strain 14 as indicated. The transgenic mice were obtained by microinjection of a purified H-2K/c-*myc* fragment into the pronuclei of fertilized eggs derived from a (C57bl/6 × SJL/J) F_1 hybrid mated to identical males (43). The H-2K/c-*myc* construct contained the human c-*myc* proto-oncogene genomic sequences (*Xho*I–*Eco*RI 5.5-kilobase fragment of the px-AHM plasmid [40]) under the control of the 5′ regulatory sequences (including the promoter) of the H-2K^b gene (30). Partial hepatectomy and sham operations were performed as described for rats (22). For experiments with cycloheximide (CHX), 4-month-old nontransgenic and H-2K/c-*myc* mice were injected with 50 mg of CHX per kg dissolved in water (37).

RNA isolation. Total RNA was extracted from livers by using LiCl (4), and the amount of RNA was determined by measuring A_{260}. All RNA samples were examined by minigel electrophoresis before being used for hybridization.

S1 nuclease analysis. Five different single-stranded, uniformly radiolabeled probes prepared in M13 vectors were used for S1 nuclease analysis. The preparation and use of mouse c-*myc* exon 1 (see diagram in Fig. 1), human c-*myc* exon 1 (see diagram in Fig. 3), H-2K, and the β2m probes have already been described (43). A mouse c-*myc* exon 3 probe (kindly provided by M. Piechaczyk) was constructed by subcloning the *Sst*I-*Xho*I fragment of the mouse c-*myc* intron 2 and exon 3 into M13mp10. The radiolabeled single-stranded probe was obtained after polymerization with the Klenow fragment of DNA polymerase I and *Pvu*II digestion. The S1-protected fragment from this probe is 370 nucleotides (nt). A total of 20 to 100 μg of RNA was hybridized for 18 h with 100,000 cpm of the single-stranded, uniformly radiolabeled probes. For the three *myc* probes the hybridization buffer was 0.5 M NaCl–20 mM Tris (pH 7.5)–1 mM EDTA–75% formamide, and the incubation temperature was 58 and 52°C for hybridization with exon 1 and exon 3 probes, respectively. S1 nuclease digestion was performed as described previously (43). Samples were precipitated with ethanol, suspended in 3 μl of formamide loading buffer, and subjected to electrophoresis in a 5% acrylamide gel. Hybridization with the β2m probe was used to monitor the amounts of RNA loaded and degradation due to sample preparation. The dried gel was exposed to Kodak XAR-2 X-ray film at −70°C with Du Pont Cronex Lightning-Plus intensifying screens.

Northern blotting. RNA samples (20 μg) were separated on 1.1% formaldehyde–agarose gels and blotted to a nitrocellulose membrane. Loading was monitored by ethidium bromide staining of gels. The ^{32}P-labeled probes used for hybridization were v-*fos* DNA (14), human c-*jun* cDNA (a gift of R. Tjian [9]), and rat glyceraldehyde-3-phosphate dehydrogenase (a gift of R. Wu [59]). After hybridization for 48 h at 42°C, filters were washed four times for 5 min each at room temperature with 0.1% sodium dodecyl sulfate–2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) followed by two more washes with 0.1% sodium dodecyl sulfate–0.1× SSC for 15 min each at 50°C. The filters were exposed to Kodak XAR-2 film at −70°C with intensifying screens.

Nuclear run-on transcription. Livers were removed from adult normal mice and homogenized in a sucrose-*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) solution (without Nonidet P-40) as described previously (42). Nuclei were purified, and nascent transcripts were elongated by using [^{32}P]UTP, essentially as described without pretreatment with RNase (47). The following single-stranded probes were used: c-*myc* exon 1 (probe A), c-*myc* exon 2–3 (probe B), c-*myc* antisense exon 1 (probe C; see diagram in Fig. 2), and 1 Fos and 2 Fos (*Hin*dIII-*Eco*RI and *Xho*I-*Nco*I fragments of the murine c-*fos* gene, respectively) (see diagram in Fig. 7 [60]). Preparation of these probes has already been described (42, 43, 60). For some experiments a double-stranded c-*myc* exon 3 probe was used. This probe is an 800-nt *Cla*I-*Eco*RI fragment of human c-*myc*. Within a given series of experiments, the hybridization reaction mixtures always contained the same amount (10^7 cpm) of [^{32}P]UTP-labeled RNA. H-2 double-stranded DNA (560-base-pair *Pst*I-*Pst*I fragment of pH-2^d 1 [10]) was used as an internal standard. M13mp10 single-stranded DNA was used to monitor nonspecific hybridization. All nuclear run-on assays were done with nuclei of individual animals, and the assays were repeated at least once.

RESULTS

Steady-state c-*myc* mRNA levels and promoter usage in regenerating liver of normal mice. To examine the changes in steady-state levels of murine c-*myc* transcripts and promoter usage at the very early phase of liver regeneration, we isolated total cellular RNA from livers of mice 30 min to 8 h after either partial hepatectomy or sham operation and from unoperated mice. RNA obtained at each time was analyzed by S1 nuclease protection assay with exon 1 and 3 murine c-*myc* probes (Fig. 1). Protection assays with the murine c-*myc* exon 3 probe (Fig. 1, EX3) showed that the amounts of c-*myc* mRNA increased in the regenerating liver and reached a maximum between 1 and 2 h after partial hepatectomy at levels approximately 10-fold higher than those of unoperated controls (Fig. 1, lane C). A reproducible increase in c-*myc* mRNA abundance also occurred in livers of sham-operated mice (Fig. 1; lanes SO), but the change was much smaller (approximately two- to threefold above that of unoperated controls) than that observed in livers of partially hepatectomized mice.

The murine c-*myc* gene is expressed from two major promoters, P1 and P2, located approximately 150 nt apart (7). In most systems P2 is more active than P1 (5–7). To examine whether c-*myc* promoter usage may change during liver regeneration, we performed protection assays with a uniformly labeled, single-stranded, 530-nt probe complementary to exon 1 of the murine c-*myc* gene. The sizes of the protected fragments are 497 and 357 nt for P1- and P2-initiated transcripts, respectively (Fig. 1, diagram). In agreement with what has been previously shown for other mouse tissues (42), we found that liver c-*myc* transcripts are preferentially initiated from P2. In regenerating livers, P1 mRNA levels remained low while P2-initiated c-*myc* mRNA levels increased approximately 12-fold between 1 and 2 h after partial hepatectomy and 2-fold in sham-operated mice (Fig.

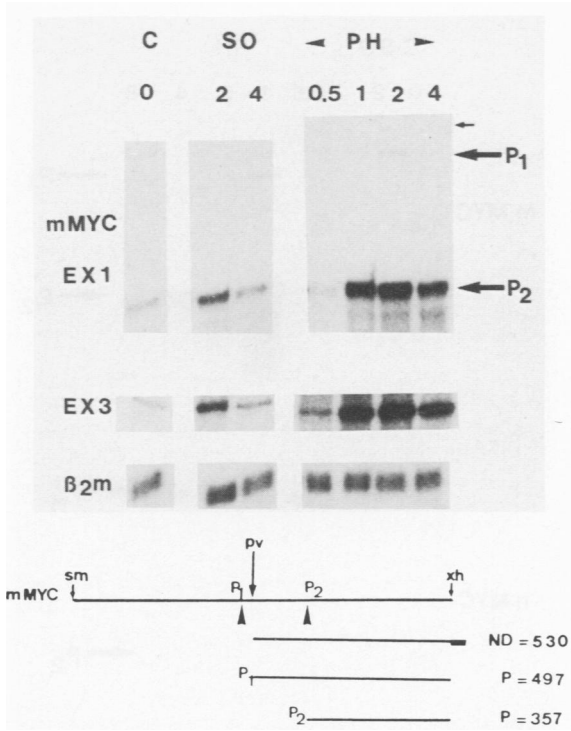

FIG. 1. S1 nuclease protection analysis of mouse c-*myc* mRNA expression during normal liver regeneration. Total RNA was prepared from livers of intact (C), sham-operated (SO), and partially hepatectomized (PH) mice, 0.5 to 4 h after the operations. RNA (20 μg) was hybridized with the mouse c-*myc* exon 1 (EX1) or exon 3 (EX3) probe and analyzed by S1 mapping as described previously (42). The diagram shows the expected sizes of the mouse c-*myc* exon 1 protected fragments: 497- and 357-nt bands corresponding, respectively, to transcripts initiated at either the P1 or P2 c-*myc* promoter. In the S1 analysis, the protected bands are indicated by heavy arrows and the position of the undigested probe (ND) is indicated by the light arrow. The β2m probe was used to control for amounts of RNA. Film exposure was 18 h for EX1 and EX3 and 40 min for β2m.

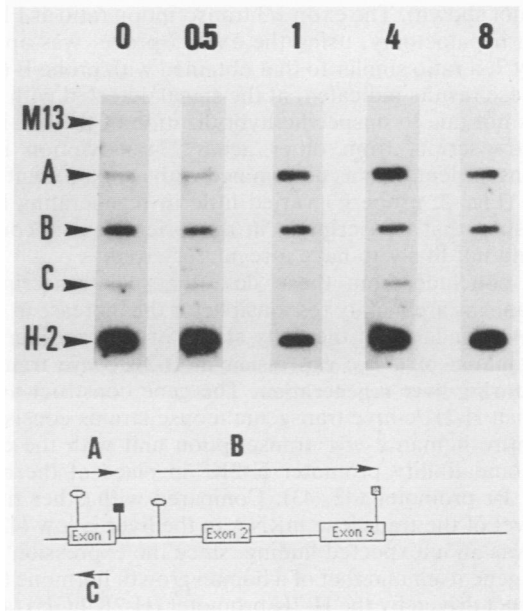

FIG. 2. Analysis of c-*myc* transcription in liver nuclei after partial hepatectomy. Nuclei were isolated from livers of intact (lane 0) and partially hepatectomized normal mice, 0.5 to 8 h after the operations. Nuclear run-on transcription was performed, and 10^7 cpm of ^{32}P-labeled RNAs were hybridized to vector M13mp10 ssDNA (M13), single-stranded c-*myc* probes in M13mp10 corresponding to the regions indicated on the diagram (A and B are probes for sense transcripts, C detects antisense transcripts), and double-stranded DNA corresponding to the 560-base-pair *Pst*I-*Pst*I fragment of pH-2^{d}1 (H-2). Hybridization with the H2 probe was used as an internal standard. Film exposure was for 3 days. Symbols: □, *Hin*dIII; ○, *Bam*HI; ■, *Bgl*II.

1, EX 1). The results indicate that promoter utilization did not change during liver growth.

c-*myc* transcription during liver regeneration. We wanted to determine whether the c-*myc* response during liver regeneration is mediated primarily at the transcriptional or post-transcriptional level. Using nuclei isolated from livers of control (nonoperated or sham-operated) mice and partially hepatectomized mice from 30 min to 8 h after the operations, we measured the elongation of in vivo-initiated transcripts by the run-on transcription method. Because the transcription of the c-*myc* gene can occur in a discontinuous manner and may involve both sense and antisense strands (47, 61), we used strand-specific probes complementary to either exon 1 or exon 2–3 in the sense orientation of the murine c-*myc* gene (Fig. 2, diagram; probes A and B, respectively) and to exon 1 in the antisense orientation (probe C). After correcting for differences in the uridine content of the A and B probes, an A/B ratio of 1 represents complete read-through of transcription complexes. Any increase in this ratio indicates that initiated transcripts have paused or are being blocked from completion. All values are expressed relative to the H-2 signal used as the internal standard.

Exon 1 transcription increased shortly after partial hepatectomy and reached a maximum at 1 h (approximately 13-fold above zero time controls [Fig. 2, probe A; Table 1]). In contrast, the levels of hybridization with the exon 2–3 probe approximately doubled during this period (Fig. 2, probe B; Table 1). Therefore, although transcriptional initiation increased after partial hepatectomy, it was compensated by a concomitant block to elongation of transcription, as indicated by the elevation in the exon 1/exon 2–3 transcription ratio in regenerating livers (Table 1, A/B ratio). Probe B has been widely used for this type of analysis (46, 48, 57), but it contains a $(CA)_{20}$ repeat sequence 5′ of exon 2 which is highly interspersed in the mouse genome. We repeated the run-on assays with normal and 1-h regenerating liver nuclei by using an exon 3 specific probe that did not contain this repeat. This fragment hybridizes to a single band in Southern blots of *Pst*I-digested mouse genomic DNA

TABLE 1. Quantitation of transcription within the c-*myc* locus during liver regeneration

DNA probe	Location in c-*myc*	Relative signal intensity[a] at time (h):				
		0	0.5	1	4	8
A	Exon 1	3.0	5.0	40	25.7	13
B	Exons 2–3	2.9	1.8	5.0	1.5	1.3
A/B		1.0	2.8	8.0	17.1	10

[a] The relative intensities of different signals were determined by densitometric scanning and are expressed in arbitrary units. The values were corrected to take into account the uridine content of each probe (A, 94 U residues; B, 585 U residues). All values are expressed relative to the H-2 signal used as an internal standard.

(data not shown). The exon 1/3 transcription ratio at 1 h after partial hepatectomy, using the exon 3 probe, was approximately 7, a ratio similar to that obtained with probe B (Table 1). These results indicate that the signal detected with probe B was not due to unspecific hybridization of the CA repeat with transcripts from other genes. Transcription in the antisense orientation as determined with an exon 1 antisense probe (Fig. 2, probe C) varied little in regenerating livers, suggesting that transcription in this orientation is constitutive and not likely to have a regulatory role.

We conclude from these data that posttranscriptional mechanisms are mainly responsible for the increase in c-*myc* mRNA abundance at the early stages of liver regeneration.

Regulation of c-*myc* expression in H-2K/c-*myc* transgenic mice during liver regeneration. The gene construct used to establish H-2K/c-*myc* transgenic mouse strains consisted of the entire human c-*myc* transcription unit with the class I histocompatibility promoter H-2K^b in place of the human c-*myc* P1 promoter (42, 43). Compared with other tissues, the level of the transgene mRNA in the liver is low (42, 43). This was an unexpected finding, since the expression of the H-2K gene itself and that of a human growth hormone (hGH) construct driven by the H-2K promoter (H-2K/hGH) (44) are high in livers of normal and H-2K/hGH transgenic mice, respectively. Therefore, we have hypothesized that the relatively low basal levels of human c-*myc* transcripts in H-2K/c-*myc* livers are due to tissue-specific posttranscriptional mechanisms that act on both murine and human c-*myc* transcripts (42). Given this background, we wanted to determine whether the transgene mRNA level would increase during liver regeneration, as shown for the endogenous gene. We also wanted to know whether the expression of the murine gene might be altered by the presence of the transgene.

The separate expression of the two c-*myc* genes in the livers of transgenic mice was analyzed by S1 nuclease protection assays with mouse and human exon 1 c-*myc* probes. To determine whether the expression of the transgene changes during liver regeneration, we used the human exon 1 probe that generates protected fragments of 450 and 354 nt for H-2K- and P2-initiated transcripts, respectively (Fig. 3, diagram). We found that in normal liver, transgene transcription was initiated mainly at the H-2K promoter and P2 transcription was very low. This was also the case in regenerating liver, but mRNA levels initiated at either site did not change after partial hepatectomy. Since in both normal and regenerating livers of transgenic mice P2-initiated transcript levels were low, we used larger amounts of RNA (100 μg) to improve the detection of P2-initiated mRNA, but even under these conditions we did not find a change in transgene mRNA levels during liver regeneration (Fig. 3, hMYC). Protection assays of liver RNA from transgenic mice (using the murine exon 1 probe) showed that the mRNA encoded by endogenous c-*myc* increased in regenerating liver of these animals (Fig. 3, mMYC). Thus, although the transgene did not respond to the signal generated by partial hepatectomy and was constitutively expressed, murine c-*myc* expression changed as expected.

To further compare the pattern of endogenous c-*myc* expression after partial hepatectomy in transgenic and nontransgenic mice, we measured the expression of the gene in two different strains of H-2K/c-*myc* transgenic animals (Fig. 4). In strain 27 mice, at 1 h after partial hepatectomy endogenous c-*myc* expression was approximately 3-fold higher than in nontransgenic animals (approximately 30-fold and 10-fold above normal, respectively). In strain 14 mice

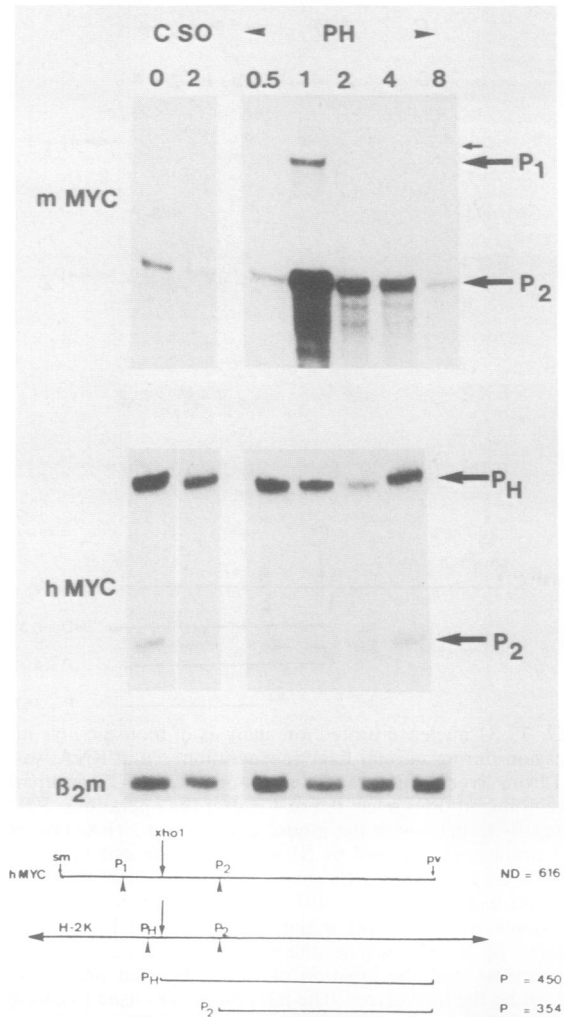

FIG. 3. S1 nuclease protection analysis of mouse and human c-*myc* mRNA expression in H-2K/c-*myc* transgenic mice during liver regeneration. Total RNA was prepared from livers of intact (C), sham-operated (SO), and partially hepatectomized (PH) H-2K/c-*myc* transgenic mice, 0.5 to 8 h after the operations. RNA (20 or 100 μg) was hybridized with the mouse c-*myc* exon 1 (mMYC) or human c-*myc* exon 1 (hMYC) probe, respectively, and analyzed by S1 mapping as described previously (42). The diagram shows the expected sizes of the human c-*myc* exon 1 protected fragments: 450- and 354-nt bands corresponding, respectively, to transcripts initiated at either the H-2 promoter (PH) or human P2 c-*myc* promoter (P2). In the S1 analysis the protected bands are indicated by heavy arrows and the position of the undigested probe (ND) is indicated by the light arrow. The β2m probe was used to control for amounts of RNA. Film exposure was for 18 h, 24 h, and 40 min for mMYC, hMYC, and β2m probes, respectively.

(43) maximal expression of endogenous c-*myc* occurred 2 h after partial hepatectomy and was approximately fourfold higher than in nontransgenic animals.

Abundance of murine c-*myc* and transgenic transcripts after CHX treatment. A plausible hypothesis to explain the transient increase in c-*myc* expression in the regenerating liver is that the synthesis of a component responsible for mRNA instability is inhibited after partial hepatectomy. Since c-*myc* mRNA can become more stable after translation blockage caused by the administration of protein synthesis inhibitors (24, 29, 36, 37), we tested whether the abundance of the

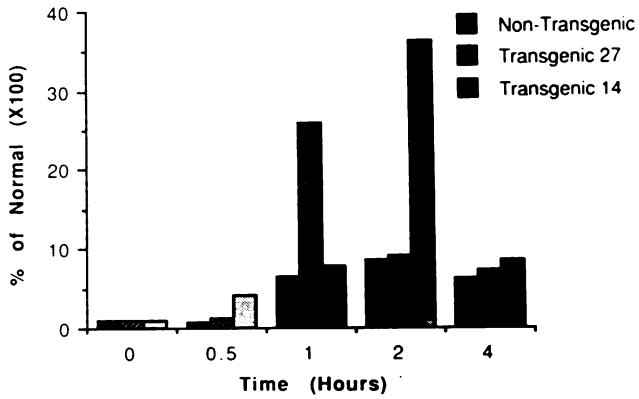

FIG. 4. Steady-state levels of endogenous c-*myc* mRNA in transgenic and nontransgenic mice. RNA was obtained from livers of nontransgenic and strains 14 and 27 H2K/c-*myc* transgenic mice from 0 to 4 h after partial hepatectomy. The data were derived by densitometric scanning of S1 protection analyses with an exon 1 probe as in Fig. 3. For each group of animals, values were normalized to the signal at time zero, which was arbitrarily set at 1.

murine and transgene mRNAs would be affected by CHX injections. A single injection of CHX caused a very large increase in c-*myc* expression in the livers of nontransgenic mice, reaching a peak about 2 h after the treatment (Fig. 5A, mMYC probe). Similar results were obtained with exon 1 and exon 3 probes, indicating that CHX caused an increase in the abundance of full-length c-*myc* transcripts (data not shown). Experiments with transgenic mice revealed that the expression of both the murine gene (Fig. 5B, mMYC probe) and the transgene (Fig. 5B, hMYC probe) increased markedly as a result of CHX injection. The change in abundance in transgene transcripts was not caused by an increase in activity of the H-2K promoter, because H-2K mRNA expression is not modified by CHX treatment (Fig. 5A, H-2K probe). We conclude that both endogenous gene and transgene mRNAs are normally subjected to degradation by a mechanism involving a labile protein. If the synthesis of this protein were inhibited after partial hepatectomy, levels of both endogenous and human c-*myc* transcripts would be expected to increase in the regenerating liver. However, since only the murine gene was modulated during liver regeneration, we conclude that the growth signal generated by partial hepatectomy does not operate primarily through this type of mechanism.

Expression of c-*fos* and c-*jun* in nontransgenic and transgenic mice during liver regeneration. We measured c-*fos* expression in regenerating mouse liver during the first day after partial hepatectomy. Expression increased 30 min after the operation, reached a maximum at 1 h (approximately 20- to 30-fold above the level in sham-operated animals), and returned to normal by 2 h (Fig. 6). c-*fos* expression at 1 h was much lower in transgenic mice than in nontransgenic animals. Similar observations were made with strain 14 H-2K/c-*myc* transgenic mice (data not shown).

Parallel expression of c-*fos* and c-*jun* has been demonstrated in a number of systems (45, 51, 54). We therefore wanted to examine whether the timing of c-*jun* expression during liver regeneration was similar to that of c-*fos*. In nontransgenic mice, the c-*jun* mRNA level increased rapidly during liver regeneration (Fig. 6) and reached a maximum at 1 h (approximately 40-fold above basal levels). Similar to c-*fos*, c-*jun* mRNA expression returned to normal within 2 h after partial hepatectomy. In transgenic mice there was an increase in c-*jun* expression after partial hepatectomy, but the extent of the change was far lower than in nontransgenic animals (Fig. 6).

We next investigated whether c-*fos* and c-*jun* transcription

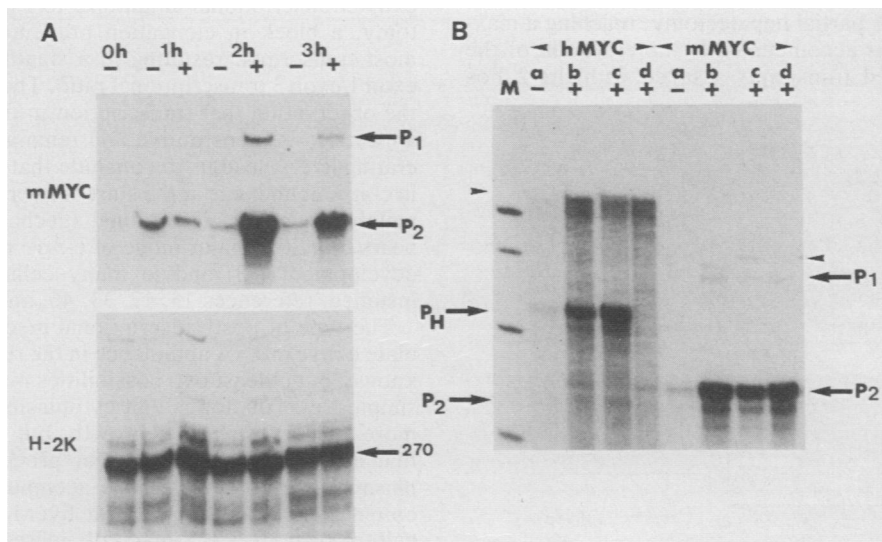

FIG. 5. S1 nuclease protection analysis of mouse and human c-*myc* mRNA after CHX treatment. (A) RNA was prepared from livers of nontransgenic mice injected with either saline (−) or 50 mg of CHX per kg (+) and killed 1 to 3 h later. RNA (20 μg) was hybridized with the mouse c-*myc* exon 1 (mMYC) probe and analyzed by S1 mapping. The P1 and P2 protected fragments are described in the legend to Fig. 1. The same samples were hybridized with the H-2K probe, whose protected fragment is 270 nt long (probe H-2K, bottom). Film exposures were 7 and 3 h, respectively. (B) RNA was prepared from livers of H-2K/c*myc* transgenic (a, b, and c) or nontransgenic (d) mice injected with either saline (−) or 50 mg of CHX per kg (+) and killed 2 h later. RNA (20 μg) was hybridized with human (hMYC) or mouse (mMYC) c-*myc* exon 1 probe and analyzed by S1 mapping. The PH, P1, and P2 (murine and human) protected fragments are as described in the legends to Fig. 1 and 3. The arrowheads indicate the positions of the undigested probe. Lane M contains pBR322 *Hpa*II fragments as molecular size markers. Film exposure was for 8 h.

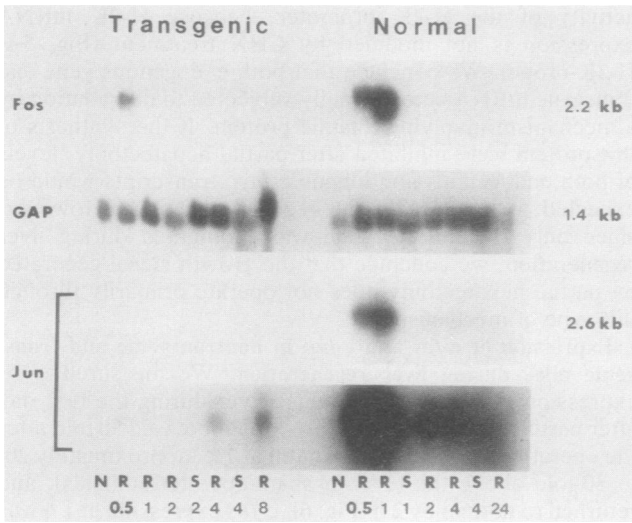

FIG. 6. Northern analysis of c-*fos* and c-*jun* expression in transgenic and nontransgenic mice during liver regeneration. RNA (20 μg) prepared from livers of nonoperated (N), sham-operated (S), and partially hepatectomized (R) normal or transgenic mice from 0.5 to 24 h after the operations was hybridized with v-*fos* (Fos), rat glyceraldehyde-3-phosphate dehydrogenase (GAP), and c-*jun* (Jun) probes as described in Materials and Methods. This filter was sequentially probed in the order c-*jun* (24- and 66-h autoradiographic exposures shown), v-*fos* (2-h exposure), and then glyceraldehyde-3-phosphate dehydrogenase (2.5-h exposure). kb, Kilobases.

would change in the regenerating liver. We performed nuclear run-on transcription assays for c-*fos* and c-*jun* with nuclei isolated from normal and regenerating livers of nontransgenic mice. Because c-*fos* transcription can be regulated by pausing (21), we used probes that recognize exon 1 and exon 2–4 transcripts (Fig. 7, 1 Fos and 2 Fos probes). Initiation of c-*fos* transcription (as measured with the 1 Fos probe) increased after partial hepatectomy, reaching a maximum at 1 h, and was accompanied by an elevation of the amounts of completed transcripts assayed with the 2 Fos

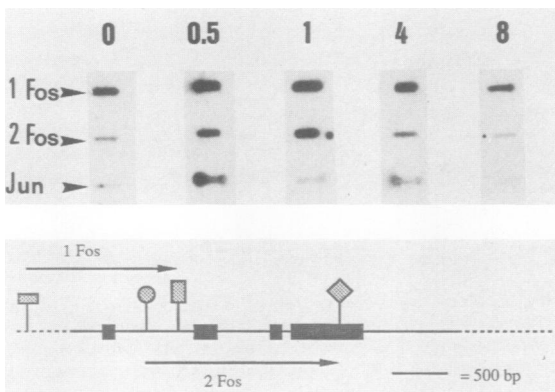

FIG. 7. Analysis of c-*fos* and c-*jun* gene transcription in normal liver nuclei after partial hepatectomy. Nuclei were isolated from livers of intact (lane 0) and partially hepatectomized mice, 0.5 to 8 h after the operations. Nuclear run-on transcription was performed, and 10^7 cpm of ^{32}P-labeled RNAs were hybridized to murine c-*fos* DNAs corresponding to the c-*fos* regions indicated on the diagram and human c-*jun* double-stranded cDNA. Film exposure was for 3 days. Symbols: ▭, *Hin*dIII; ●, *Xho*I; ▮, *Eco*RI; ◆, *Nco*I. Abbreviation: bp, base pairs.

TABLE 2. Quantitation of transcription within the c-*fos* locus during liver regeneration

DNA probe	Location in c-*fos*	Relative signal intensity[a] at time (h):				
		0	0.5	1	4	8
1 Fos	Exon 1	20.7	33.3	81	24.1	14.2
2 Fos	Exons 2–4	2.5	6.5	28	4.2	2
A/B		8.3	5.1	2.7	5.7	7.1

[a] The relative intensities of different signals were determined by densitometric scanning and are expressed in arbitrary units. The values were corrected to take into account the uridine content of each probe (1 Fos, 170 U residues; 2 Fos, 395 U residues). All values are expressed relative to the H-2 signal used as an internal standard.

probe (Fig. 7). The exon 1/exon 2–4 transcript ratio decreased transiently with the lowest level found at 1 h after partial hepatectomy (Table 2). Thus, the c-*fos* transcriptional elongation block which exists in the normal liver was partially released during the early stages of liver regeneration. c-*jun* transcription increased in the first 30 min after partial hepatectomy (Fig. 7), but, for both c-*fos* and c-*jun*, the transcriptional increase was small in comparison with the large changes in cytoplasmic steady-state mRNA detected at the same time. Thus, both transcriptional and posttranscriptional mechanisms are involved in the regulation of the expression of c-*fos* and c-*jun* at the early stages of liver regeneration.

DISCUSSION

We show that at the start of mouse liver regeneration, the transient changes in steady-state levels of c-*myc* mRNA are regulated mainly by posttranscriptional mechanisms. For c-*fos* and c-*jun*, the increase in mRNA abundance depends on both transcriptional and posttranscriptional regulation.

Run-on assays with nuclei from normal and regenerating livers showed that although there was a marked increase in c-*myc* transcriptional initiation 1 to 2 h after partial hepatectomy, a block in elongation prevented the completion of most transcripts, resulting in a significant elevation of the exon 1/exon 3 transcriptional ratio. These findings, as well as the observation that transcription in the antisense direction appears to be constitutive and remained constant in regenerating livers, lead us to conclude that the transient increase in c-*myc* abundance at the start of liver regeneration depends mainly on posttranscriptional mechanisms. This also appears to be the major mode of c-*myc* regulation during liver development (43) and in many cells in culture (see, for instance, references 15, 17, 33, 46, and 48).

The type of posttranscriptional mechanism that may regulate c-*myc* mRNA abundance in the regenerating liver is not known, but at least two possibilities may be considered. The simplest explanation is that cytoplasmic transcripts become more stable during liver growth, but it is also conceivable that more efficient intranuclear processing or nucleocytoplasmic transport may cause accumulation of cytoplasmic c-*myc* mRNA (1, 38). In murine liver in vivo, as in many cell culture systems, CHX markedly increases the c-*myc* mRNA abundance, as first shown by Makino et al. (37) for rats and by the data on mice presented in this paper. These findings, and the recent description of a destabilizing factor that acts on c-*myc* and c-*myb* mRNAs in vitro and is not present in extracts of cells treated with protein synthesis inhibitors (11), are consistent with the hypothesis that the synthesis of a protein that destabilizes c-*myc* transcripts decreases during liver regeneration. However, our data with transgenic mice

do not support this view. Indeed, in the normal liver, the levels of both transgene and endogenous c-*myc* transcripts appear to be modulated by a destabilizing factor that depends on protein synthesis. As revealed by our CHX experiments, inhibition of protein synthesis in transgenic mice led to a 50-fold increase in the transcripts of both genes. This effect is likely to be modulated by the interaction of a labile protein with the 3′ A+U-rich untranslated region of the c-*myc* mRNA that contains the AUUUA motif (56) shown to be important for c-*myc* mRNA degradation (27). However, partial hepatectomy in transgenic mice causes an increase in the expression of the murine gene but not of the transgene. This strongly suggests that although c-*myc* mRNA stabilization in the normal liver may depend on destabilizing elements acting on 3′ untranslated sequences, a different type of posttranscriptional mechanism regulates c-*myc* mRNA abundance in the regenerating liver.

Much less is known about other potential modes of posttranscriptional regulation, such as those involving intranuclear processing and nucleocytoplasmic transport (1, 34, 38, 50). If these mechanisms are responsible for c-*myc* regulation after partial hepatectomy, they may depend on sequences located 5′ to the P2 promoter that are not present in the H-2K/c-*myc* construct. This conclusion is supported by recent data demonstrating that the human c-*myc* gene, driven by its own 5′ regulatory elements, responds to growth signals when transfected into rat fibroblasts. Similar to the regenerating liver, the c-*myc* response in this system is regulated by a posttranscriptional mechanism that does not involve mRNA stabilization (52).

Elongation blockage of c-*myc* transcription is generally considered to be a mechanism that prevents overexpression of the mRNA (18). It is puzzling that both pausing and transcriptional initiation are enhanced in the regenerating liver. Increased transcriptional initiation of c-*myc* in this growth process might be a component of a more general response of various genes that share common target sequences and can be simultaneously activated by a single regulatory factor. The specific regulation of the transcription of any one of these genes would then reside in the control of elongation steps. It is also possible that the parallel enhancement of c-*myc* transcriptional initiation and elongation blockage are somehow coordinated, perhaps through the recently described ME1a1 binding site (3) and that these transcriptional events might be linked to an intranuclear step in mRNA processing. Increased transcriptional initiation in conjunction with strong elongation blockage has also been observed during kidney growth induced by folic acid (2).

c-*fos* and c-*jun* expression levels increase almost in parallel (steady-state levels approximately 20- to 40-fold above those of sham-operated controls) during the first hour of liver regeneration. Although both c-*fos* and c-*jun* transcription levels are elevated shortly after partial hepatectomy, the increase in the c-*fos* and c-*jun* steady-state mRNA level is much higher than the transcriptional enhancement, implying that posttranscriptional mechanisms also play a role in the regulation of mRNA abundance. In the normal liver c-*fos* transcription is subjected to a strong elongation block, but, in contrast to c-*myc* regulation, there is partial release of the block shortly after partial hepatectomy. Since the product of c-*jun*, the transcriptional activator AP-1, interacts with c-*fos* (25, 31, 45, 51, 55), it is not surprising that c-*fos* and c-*jun* exhibit a similar pattern of expression at the start of liver regeneration. The expression of these genes may be an important step for the chain of events that lead to the activation of the transforming growth factor alpha autocrine growth circuit in the regenerating liver (41).

Although the abundance of H-2K/c-*myc* transgene mRNA did not increase after partial hepatectomy, the transgene, perhaps through the presence of nuclear transcripts or cytoplasmic products, appears to enhance the expression of the endogenous c-*myc* in regenerating livers. This has also been shown in livers of nonoperated H-2K/c-*myc* transgenic mice (44). However, the presence of the transgene has an opposite effect on c-*fos* and c-*jun* expression after partial hepatectomy. Expression of these genes was considerably lower in the regenerating liver of transgenic animals when compared with nontransgenic mice. Studies are in progress to analyze the mechanism involved in interactions between the transgene and the endogenous proto-oncogenes and to determine whether enhanced c-*myc* expression in the regenerating livers of transgenic mice partially relieves the need for c-*fos* and c-*jun* activity.

ACKNOWLEDGMENTS

The c-*jun* probe was kindly provided by Robert Tjian. We thank J. Tso, Xiao-Hong Sun, and Ray Wu for the glyceraldehyde-3-phosphate dehydrogenase probe. We thank Agnes Lavenu for discussions and Natalie Pacheco for her help in preparing the manuscript.

This work was supported by grants 95 52 25 from the Centre National de la Recherche Scientifique and BAP 011 75 (CD) from the European Economic Community and by Public Health Service grants CA-23226 and CA-35249 from the National Cancer Institute.

ADDENDUM IN PROOF

J. Sobczak, N. Mechti, M.-F. Tourmier, J.-M. Blanchard, and M. Duguet (Oncogene **4**:1503–1508, 1989) have recently published a study of c-*myc* and c-*fos* regulation in regenerating liver.

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