Arg-Ser-rich domain-containing proteins (SR proteins), a family of splicing factors, can regulate pre-mRNA alternative splicing in a concentration-dependent manner. Thus, the relative expression of various SR proteins may play an important role in alternative splicing regulation. HRS/SRp40, an SR protein and delayed early gene in liver regeneration, can mediate alternative splicing of fibronectin mRNA. Here we determined that transcription of the HRS/SRp40 gene is induced about 5-fold during liver regeneration, similar to the level of steady-state mRNA. We found that both mouse and human HRS promoters lack TATA and CAAT boxes. The mouse promoter region from −130 to −18, which contains highly conserved GA-binding protein (GABP) and YY1 binding sites, conferred high transcriptional activity. While GABPα/GABPβ heterodimer activated the HRS promoter, YY1 functioned as a repressor. During liver regeneration, the relative amount of GABPα/GABPβ heterodimer increased 3-fold, and YY1 changed little, which could partially account for the increase in HRS gene transcription. Interleukin-6, a critical mitogenic component of liver regeneration, was able to relieve the repressive activity of the YY1 site within the HRS promoter. The combined effect of small changes in the level of existing transcription factors and mitogenic signals may explain the transcriptional activation of the HRS gene during cell growth.

SR proteins are a conserved family of splicing factors that function both as alternative splicing regulators and basal splicing factors (1, 2). At present, eight SR proteins have been identified and characterized (2). All of these proteins share two common characteristics: one or two RNA binding domains at the amino terminus and one Arg-Ser-rich domain at the carboxyl terminus. As alternative splicing regulators, SR proteins have been shown to regulate various forms of alternative splicing decisions (15, 16). The expression of SR genes during cell growth.

In our initial studies of hepatic growth, we isolated the HRS/SRp40 (hereafter referred to as HRS) cDNA, which encodes an SR protein and is a highly induced immediate early gene in insulin-treated rat hepatoma H35 cells (14). HRS mRNA is induced as a delayed early gene during liver regeneration with peak expression in rat liver at 6–8 h and approximately 5-fold induction (14). Furthermore, HRS mRNA expression is high in developing liver, especially prior to birth, when it is elevated 100-fold relative to normal adult liver (15). We showed that HRS expression correlates with the expression of EIIIB fibronectin pre-mRNA in proliferating liver. HRS can directly bind to a purine-rich enhancer in the fibronectin EIIIB exon and mediate fibronectin EIIIB exon inclusion in vivo splicing assays (15). Changes in the level of fibronectin isoforms could play a critical role in reorganizing liver architecture during liver regeneration and development.

Distinct patterns of SR protein expression during development, cell differentiation, and cell proliferation determine alternative splicing decisions (15, 16). The expression of SR genes has been shown to be regulated at the transcriptional level (1, 17). However, the mechanism of transcriptional regulation of SR genes remains largely unknown. To understand how HRS gene expression is regulated, we analyzed the mouse HRS promoter. We found that the region from −130 to −18 of the HRS promoter contained high promoter activity in HepG2 cells. We further demonstrated that GABPα/GABPβ heterodimer and YY1 regulate HRS gene expression positively and negatively, respectively. Whereas the level of YY1 changes little during liver regeneration, the relative level of GABPα/GABPβ heterodimer increases. Interleukin-6, which confers a positive growth stimulus in liver regeneration (18), can relieve YY1 repression. The combination of these effects provides a potential mechanism for HRS gene induction during liver regeneration.

MATERIALS AND METHODS

Rat Tissue Preparation—For regenerating liver, female Fisher rats (160–220 g, Charles River) or normal C57Bl6 mice were metofane (Pitman-Moore, Inc.)-anesthetized and subjected to midventral laparotomy with approximately 70% liver (left lateral and median lobes) (19). Cell Culture and Transient Transfections—Human hepatoma HepG2 and mouse fibroblast NIH 3T3 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 2 mM l-glutamine and 100 units of penicillin and 50 units of streptomycin (Life Technologies Inc.). The transient transfection was performed by the calcium phosphate method as described (20). Cells were plated on 60-mm plates at a density of 2 × 10⁵ cells/plate and transfected with the indicated amount of reporter plasmids and expression plasmids along with 2 μg of pRSV-β-galactosidase for normalization of transfection efficiency. The DNA precipitate was incubated with the cells overnight (usually 14–16 h). The cells were harvested with 1× Reporter Lysis Buffer (Promega), and the luciferase assay was performed on a Luminat luminometer (Wallace Inc.). IL-6 was added at 50 ng/ml for 4 h where indicated.
DNA fragments were separated on an 8% native polyacrylamide gel, then blunted, and the resulting blunted pGL-5.3 was digested with BglII and KpnI fragment was ligated into pGL2-basic, the 1.5-kb fragment between BglII and digested with primer from pG7–6 (24). The amplified fragment was filled in with T4 DNA polymerase and digested with BglII and EcoRI. The resulting DNA fragment was cloned into pGL2-basic between EcoRI and BglII sites. The 5′ EcoRI site was then blunted, and the resulting blunted EcoRI fragment was cloned into the KpnI (blunted) and EcoRI site in pGL-1.5. To construct pGL-2.7, pGL-5.3 was digested with SmaI and KpnI, and the KpnI site was blunted. Then the resulting 8.7-kb fragment was ligated. Further deletions of pGL-0.4 were made using Bal31 nuclease. SolI-linearized pGL-0.4 was incubated with Bal31 nuclease at 30 °C. The reaction was stopped at 3-min intervals and then digested with BglII. The resulting DNA fragments were separated on an 8% native polyacrylamide gel, and DNA fragments were eluted into 0.5 mM NaHAc and 1 mM EDTA. After ethanol precipitation, the deletion fragments were cloned into pGL-basic between BglII and Nhel (blunted) sites.

Sequence-directed mutagenesis at GABP and YY1 sites was also performed by overlapping extension PCR. All of the mutations were verified by DNA sequence analysis.

Preparation of Nuclear Extracts—HepG2 cells were grown as described previously (22). We used β2-microglobulin as a positive control and Bluescript SK(−) as negative control.

RNA, Northern Blot, and RNase Protection Assays—The RNase protection assay was carried out by using the RPA II kit from Ambion. To prepare the riboprobe, the EcoRI–ThII fragment, which covers the first exon and putative 700 bp of 5′-flanking sequence, was cloned into the pGEM4 vector, linearized with EcoRI, and in vitro transcribed with T7 RNA polymerase by adding [32P]UTP. The labeled probe was purified on a 2% agarose gel, 0.8, 0.1% SDS, 2× SSC 2–mercaptoethanol, and 10% glycerol, without bromphenol blue. The protein concentration was determined by A280. For immunoblot, 10 μg of total nuclear proteins in each lane were separated on 8% SDS-polyacrylamide gel. The proteins were transferred onto a nitrocellulose membrane and probed with 1:1000 diluted antibodies. Horse rabbit peroxidase-conjugated goat anti-rabbit immunoglobulin secondary antibody and a chemiluminescence detection system (ECL, Amersham Corp.) were used.

Plasmid Constructs—To clone the mouse HRS promoter into pGL2, the basic, 1.5-kb fragment between −1.5 kb and +100 bp was amplified by PCR with primer 5′-CCGCTGAGATCTGGAGAGGTCCG-3′ and Sp6 primer from pG7–6 (24). The amplified fragment was filled in with T4 DNA polymerase and digested with BglII and EcoRI. The resulting DNA fragment was cloned into pGL2-basic between the BglII and Nhel (blunted) sites. To construct pGL-0.4 and −0.8, the amplified fragment was digested with BglII and Nhel or digested with EcoRI first and then filled in and digested with BglII. To construct pGL-0.8, the BglII/EcoRI-blunted fragment was ligated into pGL2-basic BglII/HindIII-blunted sites. To construct pGL-0.8, the amplified fragment was digested with EcoRI and BglII and cloned into the EcoRI and BamHI sites of pGEM4. The plasmid pGL-5.3 was constructed as follows. Mouse genomic DNA from the HRS gene phage clone was digested with EcoRI. The 5′-flanking 4.6-kb EcoRI fragment was cloned into pGEM4. The 5′ EcoRI site was then blunted, and the resulting blunt EcoRI fragment was cloned into the KpnI (blunted) and EcoRI site in pGL-1.5. To construct pGL-2.7, pGL-5.3 was digested with SmaI and KpnI, and the KpnI site was blunted. Then the resulting 8.7-kb fragment was ligated. Further deletions of pGL-0.4 were made using Bal31 nuclease. SolI-linearized pGL-0.4 was incubated with Bal31 nuclease at 30 °C. The reaction was stopped at 3-min intervals and then digested with BglII. The resulting DNA fragments were separated on an 8% native polyacrylamide gel, and DNA fragments were eluted into 0.5 mM NaHAc and 1 mM EDTA. After ethanol precipitation, the deletion fragments were cloned into pGL-basic between BglII and Nhel (blunted) sites.

The sequences of double-stranded oligonucleotides were as follows: wild type YY1, 5′-AGGACGTCCTTAAAAATTTTGTGCAACG-3′; mutant YY1, 5′-AGGACGTCCTTAAAAATTTTGTGCAACG-3′; wild type GABP, 5′-ACGCCCGGAGAGGTCCG-3′; mutant GABP, 5′-ACGCCGGAGAGGTCCG-3′; and constant 2-microglobulin did not change appreciably during this time period, whereas that of the IGFBP-1 gene was increased about 14-fold at 30 min, consistent with earlier induction of IGFBP-1 gene (14, 15). Since RNA abundance may be controlled by both transcriptional and posttranscriptional mechanisms, the transcriptional activity of the HRS gene was assessed using nuclear run-on assays (Fig. 1). Transcription of HRS was increased at 2 and 6 h posthepatectomy (about 5-fold) and not increased at 30 min, consistent with the delayed early induction of HRS (14). As a control, the transcription of β2-microglobulin did not change appreciably during this time period, whereas that of the IGFBP-1 gene, an immediate early gene in liver regeneration, was increased about 14-fold at 30 min, consistent with earlier studies (22).

Lack of TATA and CAAT Boxes in the HRS Promoter—RNase protection and primer extension assays were used to map the transcription initiation site of the mouse HRS gene (Fig. 2A), which is indicated as +1 (Fig. 2B). We sequenced a 1.5-kb upstream region of the predicted transcription initiation site with the human and mouse HRS genes (GenBankTM accession numbers are AF020307 (human HRS gene) and AF020308 (mouse HRS gene)). Based on the location of the RNA initiation site, neither human nor mouse HRS promoters appear to contain TATA or CAAT boxes. With extensive alignment analysis between the human and mouse HRS genes, two regions of strong homology were found upstream of the RNA initiation site and not in more 5′ upstream regions (Fig. 2B). Two regions of identity contained a putative YY1 binding site.
Fig. 2. Localization of HRS gene RNA initiation site and mouse and human HRS promoter region sequence. A, left, RNase protection assay to map the HRS gene transcription initiation site. The RNase protection probe is indicated at the bottom, and described under “Materials and Methods.” The sequencing ladder shown is of HRS-LF rat cDNA primed with GATTACGTCTAGACCGGC (24). The RNA used was from NIH 3T3 cells, and yeast tRNA served as control. Right, primer extension analysis of RNA from regenerating mouse liver at indicated times posthepatectomy (41). The sequencing ladder is indicated at the bottom, and the nonconserved Sp1 and reverse GABP sites are boxed, and the conserved YY1 and GABP sites in the mouse promoter that were incompletely conserved in the human gene.

The Region of the HRS Promoter between −130 and −18 bp Demonstrates High Transcriptional Activity in HepG2 Cells—We chose HepG2 cells to further analyze the transcriptional control of the HRS gene, because HRS is highly expressed in HepG2 cells, and HepG2 cells represent a fetal liver phenotype (Fig. 3A). To test the ability of the predicted region to function as a promoter, we first cloned the 0.7 kb of 5′-flanking sequence of the HRS gene which included part of the first exon into a pGL2-basic luciferase reporter vector in both sense and antisense orientations (Fig. 3B). As shown (Fig. 3B), only sense pGL-0.8kb showed high luciferase activity, indicating that this region contains the HRS promoter and that the HRS promoter is orientation-dependent (comparing pGL-0.8 and pGL-r0.8). To further examine HRS promoter activity, we cloned up to 5 kb of 5′-flanking sequence and performed a series of deletion mutations. There was no significant change in promoter activity when the deletion extended from −5.3 kb to −130 bp, suggesting that there are no major regulatory elements(s) in this region, at least in HepG2 cells. When the deletion was extended to −60 bp, the promoter activity dropped about 2.5-fold, which could be explained by loss of the upstream antisense GABP site and predicted Sp1 site (see Fig. 2B). Further deletion (to −56 bp) virtually abolished HRS promoter activity. These data predict that the sequences between −130 and −18 bp of the HRS promoter contain critical regulatory element(s), consistent with the high sequence homology of this region. Using these deletion constructs in transiently transfected mouse NIH 3T3 fibroblasts, similar results were obtained, implying that this regulation was not limited to liver cells (data not shown).

Regulation of HRS Promoter by YY1 and GABP—The −130/100 promoter construct (pGL-0.1) exhibited significant promoter activity in transfected cells. Sequence analysis revealed conserved YY1 and GABP binding sites in this region of the HRS promoter. We analyzed these two binding sites in further detail. YY1 is a zinc finger transcription factor that was initially identified as a transcriptional repressor of the P5 promoter. YY1-mediated repression can be converted to activation in the presence of adenoviral protein E1A (30–32). Depending on the promoter context and cell type, YY1 can be either a transcriptional activator or repressor (33–35). To test how YY1 regulates the HRS promoter, the YY1 binding site was mutated, with the CC changed into AA (Fig. 4A). Previous studies of the P5 promoter showed that this mutation abolishes YY1 regulatory activity (27). This mutation increased HRS promoter activity about 3-fold, suggesting that the YY1 site confers transcriptional repression of the HRS promoter in HepG2 cells. Again, this effect was not cell type-specific in that the YY1 mutation increased reporter activity by about 10-fold in transfected NIH 3T3 cells (not shown).

To confirm that HepG2 nuclear extracts contain YY1, which is able to interact with the predicted YY1 binding site in the HRS promoter, we carried out EMSAs using HepG2 cell nuclear extracts and an oligonucleotide probe corresponding to the YY1 binding site in the HRS promoter. One specific DNA-protein binding complex was detected (Fig. 4B, lane 2). This complex could be competed off by excess cold wild type probe but not by mutant oligonucleotides (lanes 3 and 4). Anti-YY1 antibody, not anti-Sp1 antibody, partly supershifted but primarily disrupted this band (lanes 5 and 6). Taken together, these data suggested that YY1 specifically binds to the YY1 binding site in the HRS promoter and functions as a transcriptional repressor.

To further demonstrate the repression of the HRS promoter by YY1 in HepG2 cells, we cotransfected the HRS promoter construct (pGL-0.1) with a pCMV-YY1 expression plasmid to
determine whether overexpression of YY1 can further repress the HRS promoter. Overexpression of YY1 efficiently repressed the HRS promoter construct (Fig. 4C). As a control, overexpression of YY1 did not affect pGL2-basic or pGL2-promoter luciferase activity. Furthermore, YY1-mediated repression was dependent on the presence of an intact YY1 binding site, because the activity of the pGL-0.1mYY1 reporter was not affected by cotransfection with pCMV-YY1. Since E1A can transform YY1 from a transcriptional repressor to an activator, we predicted that overexpression of E1A would activate the HRS promoter. As shown (Fig. 4D), overexpression of E1A activated the HRS promoter about 5-fold, and activation of the HRS promoter by E1A was abolished when the YY1 binding site was mutated. As a control, E1A did not affect pGL2-basic and pGL2-promoter luciferase activity. All of these data are consistent with the finding that YY1 functions as a repressor of the HRS promoter.

GABP is an ETS transcription factor initially isolated as a transcription factor that recognizes a site important for herpes simplex virus type I immediate early gene activation. GABP is composed of two subunits: α and β (36, 37). GABPα, which contains the DNA binding domain, interacts with GABPβ to form an active heterodimer (36–39). Initial deletion mutation studies showed that deleting part of the putative GABP binding site dramatically decreased HRS promoter activity (Fig. 3). To test the possible role of GABP in HRS promoter activity, we mutated the GABP binding site in the HRS promoter in plasmid pGL-0.1 and examined how this mutation affected HRS promoter activity in HepG2 cells. As shown (Fig. 5A), this mutation decreased basal HRS promoter activity by about 15-fold. To examine whether GABP from HepG2 nuclear extract bound to the predicted GABP site in the HRS promoter, EMSSAs were performed with an oligonucleotide probe corresponding to the HRS GABP binding site (Fig. 5B). A major shifted band was detected (lane 2). This band could be competed off by wild type but not mutant oligonucleotides. In EMSA experiments performed with antibodies specific to GABPα and -β as well as anti-Sp1 antibody, only anti-GABPα and -GABPβ antibodies (lanes 5 and 6) disrupted the DNA binding complex. These data indicated that GABP bound to the site as a heterodimer.

Together pCMV-GABPα and -β activated the HRS promoter up to 5-fold. GABPα alone slightly inhibited HRS promoter activity, and GABPβ alone slightly activated the HRS promoter (Fig. 5C). These data are consistent with the previous finding that GABPα and -β synergistically activate gene expression (38). The fact that GABPα alone slightly inhibited the HRS promoter while GABPβ alone slightly activated the HRS promoter suggested that there is a limiting amount of GABPβ in HepG2 cells.

The Expression of GABP and YY1 in Regenerating Liver—HRS is induced as a delayed early gene with approximately 5-fold transcriptional induction during liver regeneration (Fig. 1). Since both GABP and YY1 regulate HRS gene expression, we wondered whether there were changes in GABP or YY1 levels during liver regeneration that might explain the increase in HRS gene transcription. We did not detect any change in the overall binding activity or the level of YY1 protein (Fig. 6, A and B). Of note, a smaller YY1 DNA binding complex was detected in EMSAs (YY1), and a 23-kDa peptide was detected in immunoblots of regenerating liver nuclear extracts (data not shown). The ∆YY1 DNA binding complex is probably an experimental artifact, since inconsistent results were obtained from different regenerating nuclear extracts (data not shown), and a single undegraded YY1 peptide was detected in an immunoblot of total liver nuclear protein that had been immediately suspended in SDS loading buffer (Fig. 6B).

Two DNA binding complexes were detected in liver nuclear extracts when the GABP binding site was used as a probe (Fig. 6C). These two complexes corresponded to the GABPα/β heterodimer and GABPα monomer (αβ and α in Fig. 6C), because both complexes were competed off by wild type oligonucleotides and the lower band supershifted only with anti-GABPα antibody (lanes 2 and 3 in Fig. 6C). The GABPα monomer band decreased during liver regeneration by about 3-fold. An immunoblot of nuclear proteins from regenerating liver was probed with a mixture of anti-GABPα and GABPβ antibodies (Fig. 6D) indicated that an increase in the level of GABPβ by 3–8 h posthepatectomy could explain the relative increase in the GABPα/β heterodimer. As predicted by Fig. 5C, an increase in the ratio of GABPα/β heterodimer to GABPα homodimer could explain at least some of the transcriptional up-regulation of the HRS gene.
The Effect of YY1, GABP, and C/EBPβ on the Net Activation of the HRS Promoter—Like E1A, C/EBPβ may transform YY1 from a dominant repressor into a transcriptional activator (40). This activity is dependent on an interaction among C/EBPβ, YY1, and the DNA binding site. We showed that C/EBPβ protein expression increases in the regenerating liver in a time course that is consistent with the transcriptional activation of the HRS gene (21) and have found a decrease in HRS expression in CEBPβ−/− livers posthepatectomy (41). However, little direct effect of C/EBPβ on HRS promoter constructs was observed in transfected HepG2 cells. In transfected NIH 3T3 cells, C/EBPβ was partially able to relieve YY1-mediated repression (not shown). The complex posttranscriptional regulation of C/EBPβ in HepG2 cells may explain the inability to demonstrate a positive effect of CEBPβ on HRS promoter reporters (42).

IL-6 mediates an early signal that is required for normal liver regeneration (18), and IL-6 is able to activate intracellular signals via both STAT and mitogen-activated protein kinase pathways. HRS mRNA expression is reduced by about 2-fold from 4–16 h posthepatectomy in IL-6−/− livers (not shown). IL-6 treatment of transfected HepG2 cells eliminated YY1-mediated repression, and this effect was dependent on an intact YY1 element (Fig. 7).

**DISCUSSION**

The region from −130 to −18 bp of the HRS promoter had high transcriptional activity. Deletion of the region from −130 to −60 bp reduced promoter activity by 2.5-fold and could be explained by the loss of an antisense GABP element at −130 and Sp1 element adjacent to the −59 GABP site. Sp1 has been found to potentiate GABP transcription (43). Two conserved transcription factor binding sites in this region, YY1 and GABP, strongly regulated HRS gene expression, YY1 acting as a repressor and GABP as an activator. GABP specifically bound the GABP binding site within the HRS promoter and transactivated the HRS promoter. The data are shown as -fold activity relative to the activity set at 1 of each reporter control when cotransfected with pCMV vector alone. For C and D, the data were from three different transfection assays with S.D. values shown.

**The Effect of YY1, GABP, and C/EBPβ on the Net Activation of the HRS Promoter**—Like E1A, C/EBPβ may transform YY1 from a dominant repressor into a transcriptional activator (40). This activity is dependent on an interaction among C/EBPβ, YY1, and the DNA binding site. We showed that C/EBPβ protein expression increases in the regenerating liver in a time course that is consistent with the transcriptional activation of the HRS gene (21) and have found a decrease in HRS expression in CEBPβ−/− livers posthepatectomy (41). However, little direct effect of C/EBPβ on HRS promoter constructs was observed in transfected HepG2 cells. In transfected NIH 3T3 cells, C/EBPβ was partially able to relieve YY1-mediated repression (not shown). The complex posttranscriptional regulation of C/EBPβ in HepG2 cells may explain the inability to demonstrate a positive effect of CEBPβ on HRS promoter reporters (42).

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c oxidase Vb (45, 46), and \( \gamma \)-chain gene (47). In all these genes, the GABP binding site is found close to the transcription initiation site, which lacks TATA boxes. In this promoter context, as postulated, GABP may function as both a transcription activator and a basal transcription factor (48). Most SR proteins have been shown to be highly expressed in spleen and thymus (49). Coincidentally, GABP has been implicated in activating genes such as CD18 (48, 50) and T cell receptor b (51) and is necessary for thymus-specific expression of the \( \gamma \)-chain promoter (47). It has been reported that both SC35 and 9G8 promoters contain putative GABP binding sites (52, 53). Therefore, GABP could function as a common transcription factor to enforce the high expression of SR proteins in hematopoietic tissues. However, unlike the SC35 and 9G8 promoters, which have typical TATA boxes, the HRS promoter lacks both TATA and CAAT boxes (52, 53). Distinct differences in the regulatory sequences controlling the HRS, SC35, and 9G8 genes provide a basis for differential expression.

At least in some cells (HepG2, NIH 3T3), YY1 functions as a transcriptional repressor of the HRS promoter. Mutation of the YY1 binding site in the HRS promoter decreased HRS promoter activity, and cotransfection of a YY1 expression vector with the HRS promoter reduced HRS promoter activity. YY1-mediated repression was reversed by oncoprotein E1A via an intact YY1 site. After adenovirus infection, viral proteins are produced largely due to alternative splicing of viral transcripts. It is likely that changes in relative expression of SR proteins mediate viral pre-mRNA alternative splicing (7, 54). Coincidentally, SV40 T antigen has similar activity (55), and it is possible that T antigen could also activate HRS expression. Of note, HRS protein expression is elevated in SV40-transformed human WI38 fibroblasts (56).

Since there is an excess amount of GABP\( \alpha \) subunit in liver that acts as a negative transcriptional regulator, we predicted that increased GABP\( \beta \) expression would lead to increased HRS gene expression during liver regeneration. In fact, the GABP\( \beta \)
expression increased only slightly but sufficiently to increase the relative level of GABPα/β heterodimer by 3-fold, eliminating most of the GABPα homodimer. We did not detect a change in the YY1 level during liver regeneration. Therefore, the changes in levels of GABP and YY1 appeared to explain some, but not all, of the up-regulation of the HRS gene during liver regeneration.

YY1 can interact with different cellular proteins, and its repression or activation function appears to be modulated by specific protein-protein interactions (57, 58). The activity of YY1 may be modulated by growth factor action, cytokines, and other types of cellular induction such as viral infection. For example, the interaction between YY1 and CREB is required by YY1 to repress the c-fos promoter (59), and the interaction between YY1 and CEBPβ is required to activate human papillomavirus type 18 promoter (40). The conversion of YY1 from repressor to activator by E1A appears to be dependent on a member of the coactivator family, p300/CBP (31, 60, 61). Both E1A and YY1 bind to p300 at different sites, and it is postulated that the addition of E1A to the p300-YY1 complex masks the repressor domain of YY1. p300/CBP can interact with various cellular and viral proteins such as CREB (62), p53 (63), pp90RSK (64), E1A (31, 65), T antigen (55), and the C/EBPs (66), many of which also interact with YY1.

IL-6 confers a positive mitogenic signal during liver regeneration and is able to relieve YY1 site-mediated repression of the HRS promoter. It is possible that intracellular signals generated by IL-6 and other growth factors that play a role in liver regeneration allow YY1 to interact with other transcription factors and coactivators converting YY1 into a activating factor or at least eliminate its negative effects. Immediate early genes show dramatic increases in transcription posthepatectomy, which are mediated by major changes in signal-induced transcription by serum response factor, AP-1, STAT3, and other transcription factors, which are themselves increased several fold (67). However, as with the HRS gene, the increase in transcription of delayed early genes is only a few fold and
results from small changes in the levels of preexisting transcription factors, combined with posttranslational effects of gene-fac tor-generated signals.

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Transcriptional Up-regulation of the Delayed Early Gene HRS/SRp40 during Liver Regeneration: INTERACTIONS AMONG YY1, GA-BINDING PROTEINS, AND MITOGENIC SIGNALS

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