An Alternative, Human SRC Promoter and Its Regulation by Hepatic Nuclear Factor-1α

Received for publication, June 6, 2000, and in revised form, August 16, 2000
Published, JBC Papers in Press, September 7, 2000, DOI 10.1074/jbc.M004882200

Keith Bonham‡§, Shawn A. Ritchie¶∥, Scott M. Dehm¶∥, Kevin Snyder¶, and F. Mark Boyd‡

From the ‡Cancer Research Unit, Health Research Division, Saskatchewan Cancer Agency and the Division of Oncology, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 4H4, Canada and the ¶Department of Biochemistry, University of Saskatchewan, 107 Wiggins Road, Saskatoon, Saskatchewan S7N 5E5, Canada

The SRC gene encodes the proto-oncogene pp60SRC, a tyrosine kinase implicated in numerous signal transduction pathways. In addition, the SRC gene is differentially expressed, developmentally regulated, and frequently overexpressed in human neoplasia. However, the mechanisms regulating its expression have not been completely explored. Here we describe the isolation of a new distal SRC promoter and associated exon, designated 1α, which we mapped to a position 1.0 kilobase upstream of the previously described SRC1A housekeeping promoter. Differential use of these promoters and their associated exons coupled with subsequent splicing to a common downstream exon results in c-Src transcripts with different 5′ ends but identical coding regions. Promoter analysis following transient transfections into HepG2 cells mapped the minimal 1α promoter to a region 145 bp upstream of the major transcription start site. This region contained a consensus binding site for hepatic nuclear factor-1 (HNF-1), a liver-enriched transcription factor implicated in the regulation of a number of genes in liver, kidney, stomach, intestine, and pancreas. Subsequent mobility shift assays confirmed that HNF-1α isoform was the predominant factor interacting with this region of the promoter. Mutation of the HNF-1 site resulted in a dramatic reduction in SRC promoter activity. Cotransfection studies demonstrated the promoter could be strongly transactivated by the HNF-1α isoform but not by the related HNF-1β factor. Consistent with these results, we demonstrated that transcripts originating from the SRC1α promoter display a tissue restricted pattern of expression with highest levels present in stomach, kidney, pancreas. These results indicate that SRC transcriptional regulation is much more complex than previously realized and implicates HNF-1 in both the tissue-specific regulation of the SRC gene in normal tissues and the overexpression of c-Src in certain human cancers.

SRC encodes pp60c-src, a nonreceptor tyrosine kinase implicated in numerous growth receptor-mediated signaling pathways leading to cellular proliferation, motility, and adhesion as well as differentiation and transformation (1, 2). More recently SRC and its close family members have been linked to both cell survival and vascular endothelial growth factor-mediated angiogenesis (3). Since its discovery as the homologue of the v-src transforming gene of Rous sarcoma virus, it has long been speculated that c-Src could play an important role in human cancer. Over the years activation of this kinase has been reported in many human neoplasms, especially those of the colon (4–8) but also breast (9, 10), lung (11, 12) pancreas (13, 14), and liver (15). Most recently, activating mutations in the SRC gene have been identified in a small number of advanced colon cancers (16). However, in many tumors and cell lines, a significant portion of this activation process appears to result from c-Src overexpression (7, 8). Interestingly, overexpression of normal c-Src is sufficient to transform mouse fibroblasts (17), especially in combination with receptor tyrosine kinases such as epidermal growth factor receptor (18, 19). In addition, antisense-mediated down-regulation of c-Src in the human colon cancer cell line (HCCL)HT29 and the SKOv-3 ovarian cancer cell line, both of which constitutively overexpress c-Src, results in decreased proliferation and tumor forming ability (20, 21). These data suggest that overexpression of c-Src can contribute to both transformation and tumorigenicity. Our own laboratory has recently shown that this common overexpression results from transcriptional up-regulation of the SRC gene in many HCCALs. Although the SRC gene product has been the subject of intense scrutiny, SRC transcriptional regulation in both normal and malignant cells has received relatively little attention. Whereas c-Src expression in normal tissues and cells is fairly ubiquitous, expression levels vary tremendously, and the gene is developmentally regulated (1, 2). In addition, SRC is induced at the transcriptional level during differentiation of several cell types (22–25) and during both maturation and activation of osteoclasts, where SRC function is essential (26, 27).

The SRC gene is composed of 14 exons; the first three, designated 1A, 1B, and 1C, encode the 5′-untranslated region of the mRNA. Exons 2–12 encode the protein and 3′ noncoding region (28, 29). A promoter, with all the hallmarks of a housekeeping gene (including high GC content and multiple start sites), is associated with exon 1A (29). We have shown that this promoter is regulated by members of the Sp1 family as well as

* This work was supported by a grant from the Medical Research Council of Canada and Medical Research Council of Canada/Saskatchewan Health bridging funds (to K. B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EMBL Data Bank with accession number(s) AF272982.

‡ To whom correspondence should be addressed: Cancer Research Unit, Health Research Div., Saskatchewan Cancer Agency, Saskatoon Cancer Center, 20 Campus Dr., Saskatoon, SK S7N 4H4, Canada. Tel.: 306-655-2315; Fax: 306-655-2635; E-mail: kbonham@scf.sk.ca.
∥ Supported by University of Saskatchewan College of Medicine Scholarships.

1 The abbreviations used are: HCCL, human colon cancer line; bp, base pair(s); CAT, chloramphenicol acetyltransferase; ELISA, enzyme-linked immunosorbent assay; EMSA, electrophoretic mobility shift assay; HNF, hepatic nuclear factor; RACE, rapid amplification of cDNA ends; PCR, polymerase chain reaction.
2 S. M. Dehm and K. Bonham, unpublished observations.
a novel factor, SPy, which interacts with several unusual poly-
urine-polypyrimidine tracts that are essential for full SRC
promoter activity (30). However, during the course of our stud-
ies with HCCls, we noted that many c-Src transcripts did not
originate from this promoter. We report here the isolation and
characterization of an alternative SRC promoter that is active
in a number of tumor cell lines, and we demonstrate that the
homeodomain-containing transcription factor HNF-1 is responsi-
able for regulating the expression of c-Src in a tissue-restricted
fashion.

EXPERIMENTAL PROCEDURES

Cell Cultures—The HT29, SW480, SW620, COL0201, and COL0205
(all colon cancers), HepG2 (hepatocarcinoma), T47D (breast cancer),
HTB 10 (neuroblastoma), and HTB 18 (retinoblastoma) human
cell lines were obtained from the American Type Culture Collection. HT29,
SW480, and SW620 were grown in Dulbecco’s modified Eagle’s medium,
COL0201 and 205 in RPMI 1640, HepG2 in Dulbecco’s modified Eagle’s
medium and Ham’s F-12 medium, T47D in RPMI 1640 supplemented
with 0.2 IU of bovine insulin, HTB 10 in McCoy’s medium, and HTB 18
in minimum essential medium and Earle’s salts. All cells were supple-
mented with 10% fetal calf serum, 1% penicillin/streptomycin and
maintained at 37 °C and 5% CO2.

RNA Isolation and Cloning of 5’ RACE Products—Total cellular RNA
was isolated from semi-confluent tissue culture plates as described (31)
and quantitated by \( A_{260} \). Concentration and integrity were confirmed
by formaldehyde agarose gel electrophoresis. For 5’ RACE analysis,
poly(A)+ mRNA was isolated from HT29 cells, using a mRNA purifica-
tion kit (Amersham Pharmacia Biotech, as described by the manufac-
turer. A 5’ RACE kit (Life Technologies, Inc.) was used, essentially as
described by the manufacturer, with some modifications. Briefly, 0.5 μg
of poly(A)+ mRNA was reverse transcribed using a SRC exon 2-specific
primer (5’ RACE primer 1; 5’-GGTCTGGAGGGGCAGGGAAAGCG)
complementary to sequence located 111 bp downstream of the initiating
AUG codon. Following RNAse treatment and purification, one-fifth of
the single-stranded cDNA was dc tad with terminal deoxynucleo-
tidy transferase. A fifth of this the reaction was then subjected to
an amplification using a second SRC exon 2-specific primer (5’ RACE
primer 2; 5’-TTGGCTTGGCTCTTGGTCAT) complementary to se-
quence located 26 bp downstream of the AUG codon and the 5’ RACE
abridged anchor primer supplied with the kit (35 cycles of denaturation
at 94 °C for 45 s, annealing at 58 °C for 60 s, and extension at 72 °C for
90 s). At this point a clear PCR product was then observed following agarose
gel electrophoresis. This PCR product (one-tenth volume) was then
amplified using a second

\[
S1 Analysis of Human Tumor Cell Lines—Two S1 probes specific for
 either 1a or 1A containing c-Src transcripts were generated from the
plasmids pSRC1a chimera and pSRC1a chimera linearized with StuI
or SacI respectively. Analysis was performed exactly as described for
 mapping transcription start sites (see above) except that an exon

1C-specific primer (5’-GAGTCCAGGGGTCCCATAGTGG) was used
to generate probes. Hybridization was carried out at 48 and 60 °C
overnight. Following S1 digestion, the products were resolved on a
standard DNA sequencing gel. Start sites were mapped by a direct
comparison with a sequencing ladder generated with pSRC1a chimera
and the exon 1B-specific primer.

Plasmids and Reporter Gene Constructions—The plasmids, pSRC1A
chimera and the genomic subclone pBam 4.8, containing the human
SRC promoter region, have been described previously (29). The CAT
promoter region, have been described previously (29). The CAT
reporter constructs containing a mutated version of the HNF-1
site (1-455mutSRC1a and 777mutSRC1a) were generated from the
wild type versions of the vectors using the QuikChange site directed
mutagenesis protocol (Stratagene). Sense 5’-TGGGAGACTGCTGGTC-
CTTCTGGAGGGACCTTGCAAC-3’ and antisense 5’-GTTGGAAG-
CCTGGCTCCAAAGGAAAGCCCGACTTCCCCC-3’, mutant oligonu-
cleotides (Life Technologies, Inc.) spanning the region —62 to –24 were
used for this process. Following confirmation of mutuation by sequence analysis the promoter
fragments were isolated and recloned into pCAT3-Basic.

Electrophoretic Mobility Shift Assays—HepG2 nuclear extracts were
prepared according to the method of Andrews and Fuller (33). A 298-bp
Mscl fragment covering the –145 to +152 region of the SRC1a
promoter was blunt end ligated into the Smal site of pBluescript. A 32P-
labeled probe encompassing the region –145 to +19 was then prepared
by digesting this clone with Clal and HindIII followed by an in-fill
reaction with Klenow fragment and [32P]dCTP. EMSAs were carried
out as described previously (30). Competitor double-stranded oligonu-
cleotide sequences (Life Technologies, Inc.) used in EMSAs were
SrrfHFN (5’-3’ N and 5’-GCTGGCGGTATCTTTAAGCA-3’, mHFN
(5’-GCTGGCGGTATCTTTAAGCA-3’) and a consensus HNF-1 se-
quence (34), cHFN (5’-GCTGCGGTTAATNATTAAC-3’). For competition
experiments, excess unlabeled oligonucleotides (32 pmol/μl) were
incubated with 50,000 cpm for 15 min prior to the addition of probe. For antibody experi-
ments 1 or 2 μg of Supershift anti-HNF-1a or HFN-1b (Santa Cruz
Bio) were used.

Immunoblot Analysis—Cells from semi-confluent 10-cm plates were
lysed directly in a loading buffer containing 65 mM Tris-HCl (pH 7.0),
2% (w/v) SDS, 5% β-mercaptoethanol, 10% glycerol, and 0.05% (w/v)
bromphenol blue. Following protein concentration determination using a Lowry kit (Sigma), 30 μg of total cellular protein was resolved on a 10% SDS-polyacrylamide gel. Gel transfer to nitrocellulose and membrane blocking were performed using standard procedures (7). Blots were first incubated with antibodies specific for HNF-1α, Sp1, or Sp3 (Santa Cruz) at 2 μg/ml, washed, and then subsequently probed with the appropriate secondary antibody conjugated to horseradish peroxidase (Santa Cruz) diluted 1:2000 as described (7). Membranes were incubated in chemiluminescence reagents (PerkinElmer Life Sciences) and exposed to Kodak X-OMAT Blue XB-1 film for detection.

**Transfections and CAT Assays**—All plasmids used in transfections were isolated using an EndoFree Plasmid Maxi Kit (Qiagen). In a typical transfection 0.5 μg of CAT vector and 0.5 μg of pCMVβ-gal were mixed with 6.0 μl of PLUS reagent and 4 μl of LipofectAMINE in 100 μl of OptiMEM (all reagents from Life Technologies, Inc.). For coexpression studies an extra 0.5 μg of either pBJ5-HNF-1α, pBJ5-HNF-1β, or an empty control vector were included. Following a 30-min incubation at room temperature, the DNA complexes were added directly to cells in serum-free medium (4 × 10^5 cells/well). After 3 h, serum was added to 10% in a final volume of 2 ml. Cells were harvested 48 h later with provided lysis buffer, and protein levels were determined using Bio-Rad Bradford reagent. β-Galactosidase activity was determined as described previously (30), and CAT levels were quantified using a CAT ELISA kit (Roche Molecular Biochemicals). CAT levels were corrected for transfection efficiency and were repeated at least three times in duplicate.

**RESULTS**

*Isolation of c-Src 5′ RACE Clones and Mapping of the SRC1α Promoter and Its Associated Exon*—As part of our efforts to understand the regulation of SRC in HCCLs, we carried out a series of 5′ and 3′RACE experiments using single-stranded ^32^P-labeled probes complementary to the 5′ noncoding region of c-Src mRNA (encoded by exons 1A, 1B, and 1C). Surprisingly, the protected fragments observed were frequently much shorter than expected and were consistent with mRNA species, which lacked sequence encoded by exon 1A (results not shown). To test this hypothesis, we carried out 5′ RACE using mRNA isolated from the HCCL, HT29, as described under “Experimental Procedures.” A major species of approximately 500 bp was isolated and cloned, and a number of individual constructs were sequenced (Fig. 1A). All of these clones contained sequence encoded by exons 1B, 1C, and 2 (the first coding exon). However, novel sequence data were obtained 5′ of exon 1B, indicating the presence of a new exon. None of the clones analyzed contained sequence encoded by exon 1A. Using traditional mapping methods, as well as the extensive sequence data available for this region of the genome (Chromosome 20 project, Sanger Institute, Cambridge, UK), we mapped this new exon to a position approximately 1.0 kilobase upstream of exon 1A and named it exon 1α (Figs. 1B and 2A).

To map the transcription start site(s) for this new proposed SRC promoter, we constructed a chimeric clone consisting of genomic sequence derived from exon 1α spliced in frame to exons 1B, 1C, and 2 derived from the 5′ RACE product, as described under “Experimental Procedures.” Single-stranded ^32^P-labeled probes were then synthesized from this construct and used in S1 mapping experiments. A single major transcription start site, as well as several minor sites, were identified (Fig. 2B). The start sites mapped were very similar or identical to those predicted by sequence analysis of the 5′ RACE clones and were also confirmed by RNase protection (results not shown).

**Relative SRC Promoter Usage in Human Tumor Cell Lines**—To examine the relative abundance of transcripts arising from promoters 1α and 1A, S1 probes were used in protection experiments with RNA isolated from various human cell lines (Fig. 3). Using similar S1 probes to those described above, we found both full-length protection, demonstrating SRC1α promoter usage, and a shorter protected species consistent with transcripts arising from 1A (Fig. 3A). These data demonstrate that both promoters are used in HCCLs such as HT29, WiDr, COLO201, COLO205, previously shown to constitutively overexpress c-Src (7). Interestingly, we also found the human hepatocarcinoma cell line HepG2, which also expresses similarly high constitutive levels of c-Src, also utilizes both promoters, although with a strong preference for 1α. HCCLs, which we have previously shown to express low levels of c-Src (SW620 and SW480), reflected this low expression in protection experiments, although species consistent with usage of both promoters were observed with prolonged exposures. In contrast, protection of RNA derived from T47D (breast cancer), HTB 10 (neuroblastoma), and HTB 18 (retinoblastoma) was consistent with predominant utilization of the 1A promoter. This was confirmed by repeating these experiments with S1 probes synthesized from vector pSRC1A chimera, where the 1α portion was replaced with 1A sequence (Fig. 3B). In this case full-length protection was seen with HTB 18 and HTB 10, confirming that these cell lines preferentially use the 1A promoter.

**Expression of 1α-derived c-Src Transcripts in Normal Human Tissues**—To determine whether transcripts originating from this new promoter were expressed in normal tissues, we first examined the expression pattern of c-Src using a multi-

---

**FIG. 1.** Analysis of an alternative c-Src transcript using 5′ RACE and genomic organization of the SRC promoter locus. A, a 500-bp 5′ RACE fragment (shown with an arrow in the right panel) amplified from HT29 mRNA was cloned, and several individual clones were sequenced. The sequence of the clone with the most extensive unique 5′ region is shown (left panel). The arrows mark the position of the various splice sites as determined by comparison with genomic sequence. The complementary sequence of the two c-Src-specific primers used in the generation of the 5′ RACE product are underlined. B, the position of the new 1α exon was mapped relative to the original 1A exon as described in the text. Several important restriction sites for subsequent cloning of CAT expression vectors are shown, including the SalI and Nael sites, which were used in the construction of the previously described 0.5SRC1A-CAT construct (29). Exon 1B is located some 20 kilobases downstream of the promoter locus. The entire sequence of this promoter region has been deposited in GenBankTM (accession number AF272982).
Since these data suggest that although overall c-Src mRNA expression is widespread, transcripts originating from the SRC1α promoter display a much more restricted pattern of expression. Unfortunately, because of an extremely high GC content, we have had difficulty constructing a reliable 1A-specific probe to complement these data. Analysis of SRC1α Promoter Activity Following Transient Transfection—To begin characterization of this new promoter, we constructed a series of 5’ and 3’ SRC1α promoter deletion CAT expression vectors (using pCAT-3basic, Promega). Because the SRC1α promoter appeared to be particularly well utilized in the HepG2 cells, we carried out our initial analysis in this cell line. As shown in Fig. 5, deletion from the 5’ end resulted in a series of stepwise increases in CAT levels, suggesting the presence of several upstream negative regulatory elements. Highest CAT levels were achieved with the −145SRC1α construct, which was approximately four times more active than the −2852SRC1α construct and produced CAT levels similar to that of the SRC1α promoter construct, 0.54SRC1αCAT3. However, deletion of sequences between −145 and +19 completely abolished this activity. Similarly, a 3’ deletion of the sequence up to −136 (−777Δ−136SRC1αCAT3) resulted in the complete loss of promoter activity. Thus, sequences in the −136 to +19 region were identified as critical for SRC1α promoter function. Examination of this region (Fig. 2A) revealed several consensus transcription factor binding sites including a weak Sp1 site (GGCGGG, positions −81 to −69), NF-IL6 (TKNNGNAAK, positions −13 to −5), and a site very similar to HNF-1 (GGTTAATNTTTAAG, positions −51 to −38). The presence of a HNF-1 site was particularly intriguing because factors binding to this site are known to regulate a variety of genes in tissues such as liver, kidney, stomach, intestine, and pancreas (35). Our multi-tissue RNA blots revealed specific SRC1α expression in all of these tissues (albeit at highly variable levels). In addition, it was clear from our S1 analysis (Fig. 3) that SRC1α transcripts were present in several colon cancer cell lines and were especially abundant in the hepatocarcinoma cell line, HepG2.

**HNF-1α Binds to and Regulates the Minimal SRC1α Promoter—**To identify factors interacting with the minimal SRC1α promoter we employed EMSAs using a 32P-labeled 163-bp fragment encompassing the −145 to +18 region of the promoter, as detailed under “Experimental Procedures.” As shown in Fig. 6A, one major diffuse complex was formed between this probe and a HepG2 nuclear extract. This complex formation was abolished by a 25 molar excess of a synthetic oligonucleotide encompassing the SRC1α HNF-1 like sequence (Src-HNF, −56 to −35) or by a consensus HNF-1 oligonucleotide (cHNF-1). In contrast a mutated version of the SRC1α HNF-1 oligonucleotide (mHNF) failed to compete. Because HNF-1 consists of two isoforms (HNF-1α and HNF-1β) that bind the same sequence as either homo- or heterodimers (36, 37), we carried out additional EMSAs in the presence of antibodies specific to either HNF-1α or HNF-1β. We found that the entire complex was supershifted in the presence of anti-HNF-1α. In contrast, anti-HNF-1β had little or no effect on the complex (Fig. 6A).

To further explore the role this sequence plays in regulating SRC1α in vivo, we introduced mutations into the −56 to −35 HNF-1 site in both −777SRC1αCAT3 and −145SRC1αCAT3. As shown in Fig. 6B this mutation resulted in the destruction of promoter activity following transfection into HepG2 cells. Because our S1 protection experiments demonstrated that the HTB 10 cell line did not express c-Src transcripts from the SRC1α promoter, we repeated these transfection experiments in these cells. None of the SRC1α constructs demonstrated measurable activity in HTB 10 cells, nor did nuclear extracts form a complex in EMSAs (results not shown). Western blot analysis demonstrated a complete absence of HNF-1α in the HTB 10 cell line, compared with intermediate levels in HT-29 cells and a strong signal from HepG2 cells (Fig. 6C). In contrast to this tissue-specific pattern of expression, Sp1 and Sp3 (which regulate SRC1α expression) appeared to be present at comparable levels.

Finally, we carried out cotransfection experiments using SRC1α-CAT3 constructs and the expression plasmids pBJ5-HNF-1α and pBJ5-HNF-1β. Because of the high endogenous...
HNF-1\(\alpha\) levels in the HepG2 cells, we chose to carry out these experiments in HT29 cells. Interestingly, all the SRC\(1\alpha\)-CAT3 constructs had much lower activity in this cell line relative to both pSV2CAT and 0.54SRC1A-CAT3. Nevertheless, as shown in Fig. 7, the single-stranded \(^{32}\)P-labeled probe was generated from pSRC1A chimera and used in S1 analysis of various human cell lines (colon cancer unless specified otherwise) as described under “Experimental Procedures.” Lanes 1–11, yeast RNA, HT29, WiDr, COLO201, COLO205, HepG2 (hepatocarcinoma) SW480, SW620, T47D (breast carcinoma), HTB 10 (neuroblastoma), and HTB 18 (retinoblastoma), respectively. The prominent protected species at 345 bp represents full-length protection, whereas the 172-bp species is consistent with a c-Src mRNA species lacking exon 1\(\alpha\) sequence. B, S1 protection using a 1A-specific probe. A S1 probe was synthesized from the pSRC1A chimera vector in a fashion similar to that described above and used to analyze RNA isolated from various cell lines. Lanes 1–7, undigested probe, yeast RNA, HT29, HepG2, SW480, HTB 10, and HTB 18. Full-length protection is seen at 274 bp, whereas the species at 172 bp is consistent with a c-Src mRNA species lacking exon 1A. Lane M, size markers were generated from a \(^{32}\)P-labeled HpaII digest of pBluescript.

**DISCUSSION**

Although the SRC gene product has been the target of intensive study for many years, we still know surprisingly little about the transcriptional regulation of this important gene. We have isolated a new SRC promoter located just upstream of the previously described SRC1A housekeeping promoter (29, 30). Differential use of these two promoters and their associated exons, coupled with subsequent splicing to common downstream exons, results in c-Src transcripts with different 5' untranslated regions that still encode identical proteins. The SRC1a promoter lies outside the CpG island, which encompasses the housekeeping promoter, and, in contrast to the multiple start sites mapped in the 1A promoter, transcription initiates from a single major site. Several lines of evidence lead us to conclude that this new SRC promoter is regulated by HNF-1\(\alpha\). First, HNF-1\(\alpha\) regulates expression of genes mainly in the liver, kidney, stomach, pancreas, and intestinal tract (reviewed in Refs. 35 and 38). Multi-tissue mRNA blots and S1 analysis of various human cell lines confirmed SRC1a expression was restricted to many of these same tissues. Second,
regulation of this promoter in HepG2 and HT29 cells was absolutely dependent on the presence of a cis-acting element that we demonstrated was predominantly bound by HNF-1α. Third, SRC1α transcripts were absent in cell lines such as HTB 10, which do not express HNF-1α. Lastly, coexpression of SRC1α–CAT3 vectors with a HNF-1α (but not the related HNF-1β) expression vector in HT29 cells resulted in transactivation of the promoter. The observation that HNF-1β is unable to transactivate the SRC promoter is consistent with a number of reports that describe HNF-1β as a weak transactivator of other HNF-1-dependent promoters (39–42).

The dual promoter system described here is therefore likely to be involved in both basal transcription in the majority of cells and tissues (most likely from the 1A promoter) as well as during transcriptional up-regulation of SRC in specific tissues during development, differentiation, or for other specific physiological reasons. A system of dual, or even multiple promoters, is found in a number of other genes, including other SRC family members such as LCK (43), and adds considerable potential flexibility to the transcriptional regulation of a gene (44). However, specific examples of how such promoters are differentially regulated are rare. The S1 protection experiments and transcription factor expression studies described here prompts us to suggest the following working model for SRC promoter regulation: in tissues or cells where HNF-1α is present at high levels, transcription would take place predominantly from the 1A promoter, even in the presence of Sp1, because the frequent transcriptional elongation from 1α would inhibit or disrupt formation of preinitiation complexes at 1A.

An example of this scenario is seen in the HepG2 cell line where there is high HNF-1α expression and predominant expression of SRC1α, despite the presence of Sp1 at levels similar to those of other cell lines. Interestingly, we found that the SRC1α and SRC1A-CAT3 expression vectors independently possess similar activities in transfection experiments in HepG2. However, S1 data strongly indicate expression from SRC1α predominates in vivo.
In cells where there is less HNF-1α but comparable levels of Sp1 (e.g. HT-29 cells), we would expect to see relatively more expression originating from SRC1A because the preinitiation complex has an increased likelihood of forming as a result of decreased expression originating from 1α. Indeed, based on our transfection data, SRC1A is a much stronger promoter than SRC1α in HT-29 cells, but S1 data suggest that the two promoters are used at similar levels in vivo. Lastly, in cells that do not express HNF-1α, there would be exclusive expression from 1α, as is the case with the HTB 10 cell line. Clearly, future experiments using a single expression vector that contains both promoters will be a prerequisite to testing these models. Furthermore, we cannot rule out the possibility that additional factors are required for full expression of the SRC1α promoter, especially in HT29 cells. Indeed, the close physical proximity of these two SRC promoters suggests that cross-talk between factors associated with each may be possible or even likely.

Our observation that SRC expression can be regulated by a tissue-specific transcription factor such as HNF-1α raises obvious questions as to what possible roles SRC might play in these tissues? Assigning specific functions to c-Src is always difficult because of the functional redundancy associated with the large c-Src family (Ref. 45 and references therein). However, an examination of the cell types in which HNF-1 is expressed presents some possible clues: HNF-1 was originally described as a liver-specific factor involved in the regulation of genes such as albumin, α-fetoprotein, and β-fibrinogen, among others (35). HNF-1β is expressed earlier in development, has a broader range of expression (46–48) and appears to be essential for visceral endoderm differentiation (47). HNF-1α, on the other hand, is expressed at a later stage and has been implicated in the maintenance or establishment of a correct differentiation state (49, 50). In contrast to the embryonic lethal phenotype of HNF-1β knockouts (47), HNF-1α gene disruption in mice does not seriously affect correct embryonic development. Offspring, however, exhibit liver enlargement, a wasting syndrome, and severe kidney dysfunction resulting from renal proximal tubular failure (50). Both HNF-1 isoforms are expressed in specialized epithelial cells (50). For example, HNF-1 expression is particularly high in the crypts of mouse small and large intestine (51). Interestingly, high pp60c-src activity has also been reported in such crypt cells, suggesting c-Src is involved in either the proliferation or differentiation of these cells, possibly under the control of HNF-1 (52). In addition, HNF-1 expression in kidney is restricted to the proximal tubules (49); c-Src plays a key role in the regulation of the NHE3 Na/H antiporter in these proximal tubules (53) suggesting again that c-Src, under the control of HNF-1, may play a role in ion transport in the kidney and perhaps in the polarized epithelia of other tissues.

Lastly, we are particularly interested in the regulation of the SRC gene in cancer cells. It is interesting to note that many of the cancers most frequently linked to c-Src overexpression (colon, liver, and pancreas) arise from tissues in which HNF-1 activity is present. A recent report has linked the ratio of HNF-1α and HNF-1β to the histological differentiation status of hepatocellular carcinoma (54), and c-Src expression levels have in the past been linked to the differentiation status of colon cancer cell lines (55). Thus, transcriptional deregulation of the SRC gene in certain cancers and its subsequent contribution to tumorigenesis may ultimately result from changes in HNF-1 expression that in turn may result from a corruption of normal differentiation pathways during neoplasia. Results described here provide an important first step in elucidating the role HNF-1 plays in c-Src overexpression in cancer, as well as expanding our understanding of the regulation of SRC transcription under normal physiological conditions.

Acknowledgment—We thank Dr. W. Roesler (Department of Biochemistry, University of Saskatchewan) for careful reading of the manuscript and helpful suggestions.

REFERENCES

1. Thomas, S. M., and Brugge, J. S. (1997) Annu. Rev. Cell Dev. Biol. 13, 513–609
2. Biscardi, J. S., Tice, D. A., and Parsons, S. J. (1999) Adv. Cancer Res. 73, 61–119
3. Schlessinger, J. (2000) Cell 100, 293–296
4. Bolen, J. B., Veillette, A., Schwartz, A. M., DeSeau, V., and Rosen, N. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 2251–2255
5. Cartwright, C. A., Kamps M. P., Meisler, A. I., Pipas, J. M., and Eckhart, W. (1989) J. Clin. Invest. 83, 2525–2533
6. Cartwright, C. A., Meisler, A. I., and Eckhart, W. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 558–562
7. Park, J., Meisler, A. I., and Cartwright, C. A. (1993) Oncogene 8, 2627–2635
8. Irvani, S., Mao, W., Fu, L., Karl, R., Yeatman, T., Jove, R., and Coppola, D. (1998) Lab. Invest. 78, 37–47
9. Ottenhoff-Kalf, A. E., Rijken, G., van Beurden, E. A. C. M., Hennipman, A., Michels, A. A., and Staal, G. E. J. (1992) Cancer Res. 52, 4773–4778
10. Verbeek, B. S., Vroom, T. M., Adriaansen-Slot, S. S., Ottenhoff-Kalf, A. E., Geertzema, J. G. N., Hennipman, A., and Rijken, G. (1996) J. Path. 186, 383–388
11. Mosetron, K., Bjelfman, C., Hammerling, U., and Pahlman, S. (1987) Mol. Cell. Biol. 7, 4178–4185
12. Mazurenko, N. N., Kogan, E. A., Zborovskaya, I. B., and Kisseljov, F. L. (1992) Eur. J. Cancer 28, 573–577
13. Lutz, M. P., Esser, I. B., Fliesser-Kast, B. B., Vogelmann, R., Lubhs, H., Friess, H., Buchler, M. W., and Adler, G. (1998) Biochem. Cell Biol. 243, 503–508
14. Visser, C. J., Rijken, G., Woutersen, R. A., and De Weger, R. A. (1996) Lab. Invest. 74, 2–11
15. Masaki, T., Okada, M., Shiratori, Y., Rengifo, W., Matsumoto, K., Maeda, S., Kato, N., Kanai, F., Komatsu, Y., Nishio, M., and Omata, M. (1998) Hepatology 27, 1284–1290
16. Irby, R. B., Mao, W., Coppola, D., Kang, J., Loubeau, J. M., Trudeau, W., Karl, R., Fujita, D. J., Jove, R., and Yeatman, T. J. (1999) Nat. Genet. 21, 187–190
17. Larsen, C. P., Shenoy, S., Galkitskii, T., and Shallaw, D. (1995) Oncogene 10, 401–405
18. Biscardi, J. S., Belsches, A. P., and Parsons, S. J. (1998) Mol. Carcinog. 21, 261–272
19. Maa, M., Leu, T., McCarley, D., Schatzman, R., and Parsons, S. J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 6981–6985
20. Wiener, J. R., Nakano, K., Kruzelock, R. P., Bucana, C. D., Bast, R. C., Jr., and Gallick, G. E. (1999) Cancer Res. 59, 2164–2170
21. Staley, C. A., Parikh, N. U., and Gallick, G. E. (1997) Cell Growth Differ. 8, 269–274
22. Higuchi, Y., Ito, M., Tajima, M., Higuchi, S., Miyamoto, N., Nishio, M., Kusagawa, S., Tsurumode, S., Sudo, A., Katou, K., Uchida, A., and Ito, Y. (1999) Bone 25, 17–24
23. Chakraborty, M., Anderson, G. M., Chakraborty, A., and Chatterjee, D. (1993) Brain Res. 625, 197–202
24. Ge, C. E., Griffin, J., Sastre, L., Miller, L. J., Springer, T., Piwnica-Worms, H., and Roberts, T. M. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 5131–5135
25. Sorci, C., Meletti, P., Dusi, S., and Bertin, G. (1993) FEBS Lett. 327, 315–320

Fig. 7. Transactivation of the SRC1α promoter by HNF-1α but not HNF-1β. The vector −145SRC1α-CAT3 and its HNF-1α mutated counterpart −145mutSRC1α-CAT3 were transfected into HT29 cells alone or in the presence of the HNF-1 expression vectors pB5-1HNF-1α or pB5-1HNF-1β. CAT levels were then assayed by ELISA. Levels are expressed as percentages of pSV2-CAT and represent an average and SD of at least three experiments performed in duplicate.
An Alternative SRC Promoter

By guest on July 25, 2018

http://www.jbc.org/Downloaded from

26. Soriano, P., Montgomery, C. R., G., and Bradley, A. (1991) Cell 64, 693–702
27. Abu-Amer, Y., Ross, F. P., Edwards, J., and Teitelbaum, S. (1997) J. Clin. Invest. 100, 1557–1565
28. Anderson, S. K., Gibbs, C. P., Tanaka, A., Kung, H.-J., and Fujita, D. J. (1985) Mol. Cell. Biol. 5, 1122–1129
29. Bonham, K., and Fujita, D. J. (1993) Oncogene 8, 1973–1981
30. Ritchie, S., Boyt, P. M., Wong, J., and Bonham, K. (2000) J. Biol. Chem. 275, 847–854
31. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
32. Courtois, G., Baumhuter, S., and Crabtree, G. R. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 7937–7941
33. Andrews, N. C., and Faller, D. V. (1991) Nucleic Acids Res. 19, 2499
34. Tronche, F., and Yaniv, M. (1992) Bioessays 14, 579–587
35. Cereghini, S. (1996) FASEB J. 10, 267–282
36. Baumhuter, S., Courtois, G., and Crabtree, G. R. (1988) EMBO J. 7, 2485–2493
37. Mendel, D. B., Hansen, L. P., Graves, M. K., Conley, P. B., and Crabtree, G. R. (1991) Genes Dev. 5, 1042–1056
38. Hayashi, Y., Wang, W., Ninomiya, T., Nagano, H., Ohta, K., and Itoh, H. (1999) Mol. Pathol. 52, 19–24
39. Hochman, J. A., Stoiky, D., Whitaker, T. L., Hawkins, J. A., and Cohen, M. B. (1997) Am. J. Physiol. 273, G833–G841
40. Lin, B., Morris, D. W., and Chou, J. Y. (1997) Biochemistry 36, 14096–14106
41. Hu, C., and Perlmutter, B. H. (1999) Am. J. Physiol. 276, G1181–G1194
42. Vallet, V., Bens, M., Antoine, B., Levrat, F., Miquerol, L., Kahn, A., and Vandewalle, A. (1995) Exp. Cell Res. 216, 363–370
43. Takadera, T., Leung, S., Gernone, A., Koga, Y., Takihara, Y., Miyamato, N. G., and Mak, T. W. (1989) Mol. Cell. Biol. 9, 2173–2180
44. Ayoubi, T. A., and Van De Ven, W. J. (1996) FASEB J. 10, 453–460
45. Klinghoffer, R. A., Sachsenmaier, C., Cooper, J. A., and Soriano, P. (1999) EMBO J. 18, 2459–2471
46. Ott, M. O., Rey-Campos, J., Cereghini, S., and Yaniv, M. (1991) Mech. Dev. 36, 47–58
47. Coffinier, C., Thepot, D., Babinet, C., Yaniv, M., and Barra, J. (1999) Development 126, 4785–4794
48. Coffinier, C., Barra, J., Babinet, C., and Yaniv, M. (1999) Mech. Dev. 89, 211–213
49. Blumenfeld, M., Maury, M., Chouard, T., Yaniv, M., and Condamine, H. (1991) Development 113, 589–599
50. Pontoglio, M., Barra, J., Hadchouel, M., Doyen, A., Kress, C., Bach, J. P., Babinet, C., and Yaniv, M. (1996) Cell 84, 575–585
51. Serfas, M. S., and Tyner, A. L. (1993) Am. J. Physiol. 265, G506–G513
52. Cartwright, C. A., Mamajewalla, S., Skolnick, S. A., Eckhart, W., and Burgess, D. R. (1993) Oncogene 8, 1033–1039
53. Tsuganezawa, H., Preisig, P. A., and Alpern, R. J. (1998) Kidney Int. 54, 394–398
54. Wang, W., Hayashi, Y., Ninomiya, T., Ohta, K., Nakabayashi, H., Tamaki, T., and Itoh, H. (1998) J. Pathol. 184, 272–278
55. Weber, T. K., Steele, G., and Summerhayes, I. C. (1992) J. Clin. Invest. 90, 815–821
An Alternative, Human SRC Promoter and Its Regulation by Hepatic Nuclear Factor-1 α
Keith Bonham, Shawn A. Ritchie, Scott M. Dehm, Kevin Snyder and F. Mark Boyd

J. Biol. Chem. 2000, 275:37604-37611.
doi: 10.1074/jbc.M004882200 originally published online September 7, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M004882200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 55 references, 17 of which can be accessed free at http://www.jbc.org/content/275/48/37604.full.html#ref-list-1