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Cloning, Genomic Organization, and Tissue Distribution of Human Ssf-1

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During the screening of a human placenta cDNA library, realized in order to isolate the $P2Y_{11}$ coding sequence, an unrelated cDNA was cloned. We identified a 1422 bp open reading frame encoding a human protein displaying 40% amino acid identity with the *Saccharomyces cerevisiae* Ssf-1, a protein involved in the second step of mRNA splicing. Sequencing of the corresponding genomic DNA showed that the gene encoding human Ssf-1 is located upstream to the $P2Y_{11}$ gene on chromosome 19p31. Comparison of the cDNA and genomic DNA sequences revealed that the human Ssf-1 gene is split into 12 exons. Northern blotting experiments showed that the 1.7 kb Ssf-1 mRNA presents an ubiquitous tissue expression. We also show that, in HL-60 human promyelocytic leukemia cells, Ssf-1 mRNA is rapidly upregulated following a treatment by granulocyte-colony stimulating factor and dibutyryl-cyclicAMP, two agents known to induce the granulocytic differentiation of these cells. © 2000 Academic Press

Key Words: **human Ssf-1; yeast; splicing; mRNA; HL-60.**

The splicing of pre-mRNAs occurs via two phosphoribosyl transfer reactions commonly referred to as the first and the second steps. In the first step, the 5′-exon is cleaved concomitantly with the production of a lariat intermediate. In the second step, the 3′-splice site is cleaved, the exons are ligated, and the intron is released as a lariat product. This process is catalysed by a large multicomponent ribonucleoprotein complex called the spliceosome. In contrast to the progress in identifying proteins required for early stages in spliceosome assembly, little is known about the proteins involved in catalytic step II, especially in mammals. Genetic screening in *Saccharomyces cerevisiae* have led to the identification of 4 proteins specifically required for the second step of splicing: Prp16, Prp17, Prp18 and Slu7 (1). The cDNAs encoding the human counterparts of these proteins have been cloned, and present between 30 and 40% of amino acid identity with their yeast orthologs. These human proteins can partially complement yeast knockout strains (2–5). In yeast, another second-step splicing factor, Ssf-1, is absolutely required for the splicing of actin pre-mRNA *in vitro* and acts during the Prp16-dependent step (6). Using mRNA differential display to identify the genes whose transcription is downregulated during apoptosis of the human hepatocellular carcinoma cell line Hep3B, Ahn *et al.* isolated a partial cDNA displaying a significant sequence homology with the yeast Ssf-1 gene (7).

In a completely unrelated study concerning the P2Y receptors which mediate the actions of extracellular nucleotides, we cloned the human $P2Y_{11}$ receptor, which is activated by ATP and positively coupled to the phospholipase C and the adenylyl cyclase pathways (8). We now report that, during that study, we isolated some cDNA clones from a human placenta library which contained an open reading frame presenting 40% amino acid identity with the yeast Ssf-1 sequence. Furthermore, we describe the organization of the human Ssf-1 gene and show that the Ssf-1 mRNA expression in HL-60 human promyelocytic leukemia cells is upregulated by agents known to induce their granulocytic differentiation.

MATERIALS AND METHODS

Materials. Trypsin was from Flow Laboratories (Bioggio, Switzerland). Culture media, fetal calf serum (FCS), restriction enzymes and T4 DNA ligase were purchased from GIBCO BRL (Merelbeke, Belgium). [α^{32}P]dATP (800 Ci/mmol) was from Amersham (Ghent, Belgium). G-CSF and dbcAMP were from Sigma Chemical Co (St Louis, MO). RNeasy kit was from Qiagen (Leusden, The Netherlands). The HL-60 human promyelocytic leukemia cell line was obtained from the American Type Culture Collection (Rockville, MD). The human genomic DNA library was from Stratagene (La Jolla, CA). The human placenta cDNA library was generously given by Pr. P. Chambon (Strasbourg, France). Multiple human tissue Northern blots (MTN) were from CLONTECH (Palo Alto, CA).

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   1  AGGAGGCCTCGTGGAGGACACAGCAGCATGGGACAGTCAGGGAGGTCCCGGCACCAGAAG      60
   1                                M  G  Q  S  G  R  S  R  H  Q  K      20

  61  CGCGCCCGCGCCCAGGCGCAGCTCCGCAACCTCGAGGCCTATGCCGCGAACCCGCACTCG     120
  21   R  A  R  A  Q  A  Q  L  R  N  L  E  A  Y  A  A  N  P  H  S      40

 121  TTCGTGTTCACGCGAGGCTGCACGGGTCGCAACATCCGGCAGCTCAGCCTGGACGTGCGG     180
  41   F  V  F  T  R  G  C  T  G  R  N  I  R  Q  L  S  L  D  V  R      60

 181  CGGGTCATGGAGCCGCTCACTGCCAGCCGTCTGCAGGTTCGTAAGAAGAACTCGCTGAAG     240
  61   R  V  M  E  P  L  T  A  S  R  L  Q  V  R  K  K  N  S  L  K      80

 241  GACTGCGTGGCAGTGGCTGGGCCCCTCGGGGTCACACACTTTCTGATCCTGAGCAAAACA     300
  81   D  C  V  A  V  A  G  P  L  G  V  T  H  F  L  I  L  S  K  T     100

 301  GAGACCAATGTCTACTTTAAGCTGATGCGCCTCCCAGGAGGCCCCACCTTGACCTTCCAG     360
 101   E  T  N  V  Y  F  K  L  M  R  L  P  G  G  P  T  L  T  F  Q     120

 361  GTGAAGAAGTACTCGCTGGTGCGTGATGTGGTCTCCTCACTGCGCCGGCACCGCATGCAC     420
 121   V  K  K  Y  S  L  V  R  D  V  V  S  S  L  R  R  H  R  M  H     140

 421  GAGCAGCAGTTTGCCCACCCACCCCTCCTGGTACTCAACAGCTTTGGCCCCCATGGTATG     480
 141   E  Q  Q  F  A  H  P  P  L  L  V  L  N  S  F  G  P  H  G  M     160

 481  CATGTGAAGCTCATGGCCACCATGTTCCAGAACCTGTTCCCCTCCATCAACGTGCACAAG     540
 161   H  V  K  L  M  A  T  M  F  Q  N  L  F  P  S  I  N  V  H  K     180

 541  GTGAACCTGAACACCATCAAGCGCTGCCTCCTCATCGACTACAACCCCGACTCCCAGGAG     600
 181   V  N  L  N  T  I  K  R  C  L  L  I  D  Y  N  P  D  S  Q  E     200

 601  CTGGACTTCCGCCACTATAGCATCAAAGTTGTTCCTGTGGGCGCGAGTCGCGGGATGAAG     660
 201   L  D  F  R  H  Y  S  I  K  V  V  P  V  G  A  S  R  G  M  K     220

 661  AAGCTGCTCCAGGAGAAGTTCCCCAACATGAGCCGCCTGCAGGACATCAGCGAGCTGCTG     720
 221   K  L  L  Q  E  K  F  P  N  M  S  R  L  Q  D  I  S  E  L  L     240

 721  GCCACGGGCGCGGGGCTGTCGGAGAGCGAGGCAGAGCCTGACGGCGACCACAACATCACA     780
 241   A  T  G  A  G  L  S  E  S  E  A  E  P  D  G  D  H  N  I  T     260

 781  GAGCTGCCTCAGGCTGTCGCTGGCCGTGGCAACATGCGGGCCCAGCAGAGTGCAGTGCGG     840
 261   E  L  P  Q  A  V  A  G  R  G  N  M  R  A  Q  Q  S  A  V  R     280

 841  CTCACCGAGATCGGCCCGCGGATGACACTGCAGCTCATCAAGGTCCAGGAGGGCGTCGGG     900
 281   L  T  E  I  G  P  R  M  T  L  Q  L  I  K  V  Q  E  G  V  G     300

 901  GAGGGCAAAGTGATGTTCCACAGTTTTGTGAGCAAGACGGAGGAGGAGCTGCAGGCCATC     960
 301   E  G  K  V  M  F  H  S  F  V  S  K  T  E  E  E  L  Q  A  I     320

 961  CTGGAAGCCAAGGAGAAGAAGCTGCGGCTGAAGGCGCAGAGGCAGGCCCAGCAGGCCCAG    1020
 321   L  E  A  K  E  K  K  L  R  L  K  A  Q  R  Q  A  Q  Q  A  Q     340

1021  AATGTGCAGCGCAAGCAGGAGCAGCGGGAGGCCCACAGAAAGAAGAGCCTGGAGGGCATG    1080
 341   N  V  Q  R  K  Q  E  Q  R  E  A  H  R  K  K  S  L  E  G  M     360

1081  AAGAAGGCACGGGTCGGGGGTAGTGATGAAGAGGCCTCTGGGATCCCTTCAAGGACGGCG    1140
 361   K  K  A  R  V  G  G  S  D  E  E  A  S  G  I  P  S  R  T  A     380

1141  AGCCTGGAGTTGGGTGAGGACGATGATGAACAGGAAGATGATGACATCGAGTATTTCTGC    1200
 381   S  L  E  L  G  E  D  D  D  E  Q  E  D  D  D  I  E  Y  F  C     400

1201  CAGGCGGTGGGCGAGGCGCCCAGTGAGGACCTGTTCCCCGAGGCCAAGCAGAAACGGCTT    1260
 401   Q  A  V  G  E  A  P  S  E  D  L  F  P  E  A  K  Q  K  R  L     420

1261  GCCAAGTCTCCAGGGCGGAAGCGGAAGCGGTGGGAAATGGATCGAGGCAGGGGTCGCCTT    1320
 421   A  K  S  P  G  R  K  R  K  R  W  E  M  D  R  G  R  G  R  L     440

1321  TGTGACCAGAAGTTTCCCAAGACCAAGGACAAGTCCCAGGGAGCCCAGGCCAGGCGGGGG    1380
 441   C  D  Q  K  F  P  K  T  K  D  K  S  Q  G  A  Q  A  R  R  G     460

1381  CCCAGAGGGGCTTCCCGGGATGGTGGGCGAGGCCGGGGCCGGGGCCGCCCAGGGAAGAGA    1440
 461   P  R  G  A  S  R  D  G  G  R  G  R  G  R  G  R  P  G  K  R     480

1441  GTGGCCTGAGCCCAAGCCGCACCGGAGCAGCGGCTGGATTGAACGCCCCAGATTGGGGCC    1500
 481   V  A  *

1501  CGAGATGTGGCCCTCGGTTTCCTTTCATAAAGGAGTTGTGTCCCCAGCCCTTCCACTCCA    1560
1561  GTAAAGAACTGAATTGGC
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FIG. 1. Nucleotide and deduced amino acid sequences of human Ssf-1.

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ySsf-1  -----MAKRRQKKRTHAQL-TPEQEQGIPKSMVIRVGQTSLANHSLNQLVKDFRQIMQPH
ySsf-2  -----MAKRRQKKRTHAQI-TPEQERDIPKSMVIRVGQTSLANHSLNQLVKDFRQIMQPH
hSsf-1  MGQSGRSRHQKRARAQAQLRNLEAYAANPHSFVFTRGCT---GRNIRQLSLDVRRVMEPL
              :::::: *::**:   *     *:*:*:  * *    : : **  * *::*:*

ySsf-1  TAIKLKERKSNKLKDFVVMCGPLGVTHLFMFTQSEKTGNVSLKIARTPQGPTVTFQVLDY
ySsf-2  TAVKLKERKSNKLKDFVVMCGPLGVTHLFMFTQSEKTGNVSLKIARTPQGPTVTFQVLDY
hSsf-1  TASRLQVRKKNSLKDCVAVAGPLGVTHFLILSKTET--NVYFKLMRLPGGPTLTFQVKKY
        ** :*: ** * *** * : *******::::::::*   ** :*: * * ***:****  *

ySsf-1  SLGRDIKKFLKRPKSLNNDDVLNPPLLVLNGFSTSKRSGEDDQDVNVEKVIVSMFQNIFP
ySsf-2  SLGRDIKKFLKRPKSLNNDDVLNPPLLVLNGFSTSKRSDEDDQDVNVEKVIVSMFQNIFP
hSsf-1  SLVRDVVSSLRRHR-MHEQQFAHPPLLVLNSFG--------PHGMHV-KLMATMFQNLFP
        ** **:   *:* : :::::  :******* *          : ::* *:: :****:**

ySsf-1  PLNPARTSLNSIKRVFMINKDRETGEISMRHYFIDIREVEISRNLKRLYKAKNNLSKTVP
ySsf-2  PLNPARTSLNSIKRIFMINKDRETGEISMRHYFIDIREVEISRNLKRLYKAKNNLSKTVP
hSsf-1  SINVHKVNLNTIKRCLLIDYNPDSQELDFRHYSIKVVPVGASRGMKKL------LQEKFP
         :*  :  **:*** ::*: : :: *: :*** * :  *  ** :*:*      * :  *

ySsf-1  NLHRKEDISSLILDHDLGAYTSESEIEDDAIVRVVDNQDVKAKHSQSLKSQRTPVEKKDN
ySsf-2  NLHRKEDISSLILDHDLGAYTSESEIEDDAIVRVVDNQDVKAKHSQTSLSQKTPVKMTDN
hSsf-1  NMSRLQDISELLA---TGAGLSESEAEPD-----------GDHNITELPQ--AVAGRGN
        *: * :*** *:     **  **** * *             *  :   *   *    *

ySsf-1  KEREKETEEEDVEMEEPKPSENLQPTPRKKAIKLTELGPRLTLKLVKIEEGICSGKVLHH
ySsf-2  EEREKGIEEEDVEMEEPKPSENSQPTPRKKAIKLTELGPRLTLKLVKIEDGICSGKVLHH
hSsf-1  MRAQ----------------------QSAVRLTEIGPRMTLQLIKVQEGVGEGKVMFH
           :                       : *::***:***:**:*:*:::*:  ***: *

ySsf-1  EFVQKSSEEIKALEKRHAAKMRLKEQRKKEQEENIAKKKAVKDAKKQRKLE-RRKAR---
ySsf-2  EFVQKSSEEIKALEKRHAAKMRLKEQRRKEQEENIAKKKAVKDAKKQRKLE-RRKAR---
hSsf-1  SFVSKTEEELQAILEAKEKKLRLKAQRQAQQAQNVQRKQEQREAHRKKSLEGMKKARVGG
         ** *: **::*: : :  *:*** **: :* :*: :*:  ::*:::: **  :***

ySsf-1  AAEGGEGQGKDDAM-----SDDESSSSDSEHY-GSVPEDLDSDLFSEVE-----------
ySsf-2  AEEQGEGQGKDGAM-----SDDGSSSSEDEHY-SDVPEDLDSDLFSEVE-----------
hSsf-1  SDEEASGIPSRTASLELGEDDDEQEDDDIEYFCQAVGEAPSEDLFPEAKQKRLAKSPGRK
        : *   *     *       **     : *::   * *    *** * :

ySsf-1  ------------------------------------------------------------ 453aa
ySsf-2  ------------------------------------------------------------ 453aa
hSsf-1  RKRWEMDRGRGRLCDQKFPKTKDKSQGAQARRGPRGASRDGGRGRGRGRPGKRVA 473aa
```

FIG. 2. Alignment of the hSsf-1, ySsf-1, and ySsf-2 amino acid sequences. The amino acid sequences of ySsf-1 (453aa; P38789), ySsf-2 (453aa; Q12153), and hSsf-1 (473aa; AJ292529) are aligned. Identical amino acids are indicated by (*) and conserved amino acids by (:). Gaps (−) were introduced to maximize the alignment.

Cloning and sequencing. A human placenta cDNA library was first screened at moderate stringency with an [α^{32}P]dATP-labeled $hP2Y_4$ receptor probe (region between the transmembrane domains 3 and 7). One of the positive clones obtained proved to be a fusion product of $hP2Y_{11}$ and a fragment of hSsf-1. This clone was used as a probe to screen the library at high stringency and isolate the complete cDNA coding for human Ssf-1. During the characterization of the genomic $P2Y_{11}$ DNA clones (8), the hSsf-1 gene was located upstream of the $P2Y_{11}$ gene. The largest genomic clone containing $hP2Y_{11}$ (17 kb in λZapII) was mapped by restriction enzyme digestions and fragments were subcloned in pBluescript. The DNA fragments were sequenced using the BigDye Terminator cycle sequencing kit (Applied Biosystems, Great Britain).

Cell culture and stimulation. HL-60 cells were maintained in RPMI 1640 supplemented with 10% FCS, 5 mM L-glutamine, 50 units/ml penicillin and 50 μg/ml streptomycin at 37°C with 5% CO_2. The HL-60 cells were then incubated in the presence or the absence of G-CSF (10 ng/ml) or dbcAMP (200 μM) for different periods of time.

Northern blot analysis. One blot containing poly(A)$^+$ RNA from 12 different human organs (2 μg of poly(A)$^+$ RNA/lane) and a blot containing total RNA extracted from differentiated or undifferentiated HL-60 cells were hybridized with a [α^{32}P]dATP-labeled human Ssf-1 probe (EcoRI/BamHI fragment containing approximately the first 1120 bp of the cDNA). After treatment or not with differentiating agents, the total RNA from HL-60 cells was extracted with the RNeasy kit, according to the manufacturer's instructions. The same quantity for each condition (10 μg/lane) of total RNA was separated by electrophoresis on a 1% agarose gel in 10 mM phosphate buffer, pH 7.0. After acridine orange staining, the RNA's were transferred to

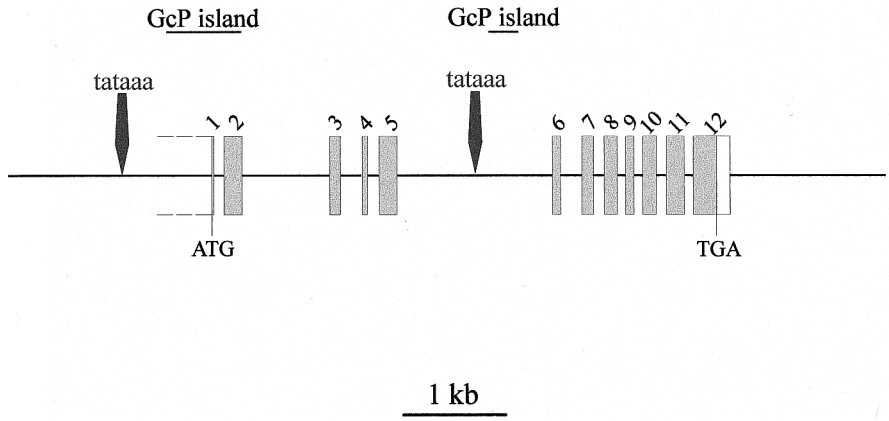

FIG. 3. Organization of the human Ssf-1 gene. Schematic representation of the Ssf-1 gene, which is split into 12 exons numbered from 1 to 12. Each box represents an exon. The beginning of exon 1 has not been identified. Gray boxes represent Ssf-1 coding regions. White boxes indicate noncoding regions. Introns are represented by lines. The position of the translation initiation (ATG) and the stop (TGA) codons are indicated, as well as GcP islands and potential TATA elements.

a nylon membrane using 20× SSC (3 M NaCl/0.3 Na citrate). The different blots were prehybridized 6 h at 42°C in a 50% formamide/0.3% SDS solution and hybridized for 18 h in the same solution supplemented with [α^{32}P]dATP-labeled human Ssf-1 probe. The final washing conditions were 0.2× SSC and 0.1% SDS at 55°C. The blots were exposed and visualized by autoradiography.

RESULTS

The human $P2Y_{11}$ receptor was first isolated from a human placenta cDNA library by screening, at moderate stringency, with a human $P2Y_4$ probe (6). During this screening, a 2385 bp chimeric cDNA clone was isolated. This clone contained an unrelated partial open reading frame located upstream of a 5′-truncated $hP2Y_{11}$ fragment. Using this chimeric clone as a probe in a new screening, we isolated a complete cDNA (1578 bp) of this unrelated sequence, which contained a 1422 bp open reading frame (Fig. 1). The deduced amino acid sequence presents 40% amino acid identity with the sequence of two *S. cerevisiae* splicing factors, Ssf-1 and Ssf-2 (Fig. 2). The homology is observed over the entire sequence.

The sequencing of a 17 kb genomic DNA clone previously isolated and containing the $P2Y_{11}$ gene (8), revealed that the hSsf-1 gene is located 5′ to the $P2Y_{11}$ gene. Comparison with the cDNA sequence of Ssf-1 revealed that this gene has a size of approximately 6 kb and is split into 12 exons (Fig. 3). Introns vary in length from 77 to 1495 bp and exons vary from 51 to 171 bp. The last exon contains 129 bp of noncoding sequence. Furthermore, all the sequences at the exon/intron boundaries are in full agreement with the canonical splice donor and acceptor sites consensus sequences (Table 1). In order to confirm these results and to identify potentials elements that control the transcription, we analysed the entire sequenced DNA using the GRAIL 2 program. This analysis confirmed the exon/intron organization. Moreover, as represented in Fig. 3, this DNA sequence presents two characteristics

TABLE 1

Exon/Intron Organization of the hSsf-1 Gene

Exon number	Exon size (bp)	5′ Exon junction	3′ Exon junction	Amino acid interrupted	Intron class
I	?	—	TCA GGG AGG **gt** aagg	—	0
II	171	gtcgccg**cag** GTT CGT	CGT CTG CAG **gt** ttgt	—	0
III	102	tcatttc**cag** GTT CGT	GTC TAC TTT **gt** gagt	—	0
IV	51	tctcctg**cag** AAG CTG	GTG AAG AAG **gt** gaga	—	0
V	171	tgtccta**cag** TAC TCG	GTG CAC AAG **gt** gggt	—	0
VI	77	ccccttc**cag** GTG AAC	CGC CAC TA **gt** gagt	Y	2
VII	108	gttcctg**cag** T AGC ATC	CTG GCC AC **gt** gagg	T	2
VIII	124	ctacaca**cag** G GGC GCG	CTC ACC GAG **gt** gagg	—	0
IX	79	gtcccct**cag** ATC GGC	AGT TTT G **gt** gagg	V	1
X	130	gttggct**cag** TG AGC AAG	GCC CAC AG **gt** ccag	R	2
XI	170	cagccca**cag** A AAG AAG	AGT GAG G **gt** atgg	D	1
XII	350	ctcccca**cag** AC CTG TTC	—		

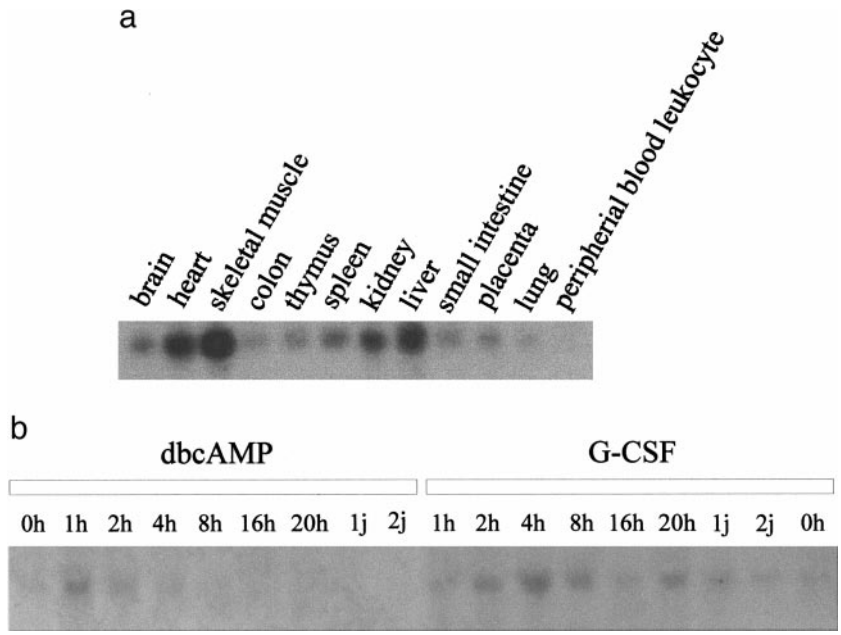

FIG. 4. Northern blot analysis of hSsf-1 messenger expression. (a) Each lane of the CLONTECH blot contains 2 μg of poly(A)$^+$ RNA from indicated human tissues. (b) Each lane of the blot contains 10 μg of total RNA from HL-60 treated for indicated times in the presence of differentiating agents, G-CSF (10 ng/ml) and dbcAMP (200 μM). Hybridization with the probe was performed as described under Materials and Methods.

typical of GpC islands located upstream to the first and the sixth exons. Two TATA elements were also identified close to these GpC islands. These results indicate the presence of two potential promoting regions. However, as described below, only one specific hSsf-1 mRNA at 1.7 kb was detected in the Northern blotting experiments.

The sequencing of the 3′ end of the genomic DNA clone revealed, on the opposite strand, the known sequence of the EIF3S4 gene, coding for the subunit 4 of the translation initiation factor eIF3 (9). Because the human EIF3S4 gene has been mapped on chromosome 19p31, between the markers D19S413 and D19S221, we can conclude that human Ssf-1 and $P2Y_{11}$ genes are also located in this region of chromosome 19.

A blot containing poly(A)$^+$ RNA of 12 human tissues was hybridized with a partial hSsf-1 cDNA probe. We observed a signal at 1.7 kb in all organs represented on the blots. Some of them present a very strong signal *i.e.* heart, skeletal muscle, kidney and liver. The messenger was less abundant in the other tissues *i.e.* brain, colon, thymus, spleen, small intestin, placenta, lung and peripheral blood cells (Fig. 4a). In HL-60 cells, this mRNA was rapidly upregulated in response to granulocyte-colony stimulating factor (G-CSF) and dibutyryl-cyclicAMP (dbcAMP) (Fig. 4b). The accumulation of Ssf-1 messenger was maximal at 4 h for G-CSF and 1 h for dbcAMP.

DISCUSSION

The complete human Ssf-1 cDNA has been identified thanks to the fortuitous isolation of a chimeric clone between Ssf-1 and $hP2Y_{11}$. This phenomenon has already been described in rare instances for other eukaryote genes (10, 11), but its significance if any remains unclear. The human sequence of Ssf-1 showed a high degree of conservation with the yeast protein, a feature characterizing other second step splicing factors. Indeed the human orthologs of Prp16, Prp17, Prp18 and Slu7 display about 40% amino acid identity with the yeast proteins (2–5). They function in step II of splicing *in vitro* in humans and can partially rescue yeast strains in which the corresponding proteins have been knocked out (3, 4). These results indicate that the mechanism of catalytic step II is highly conserved and suggest that human Ssf-1 could also be active in that process in humans. The ubiquitous expression of Ssf-1 mRNA is also consistent with a general role in pre-mRNA splicing.

Ahn *et al.* previously reported the down-regulation of Ssf-1 messengers during the PGA_2-induced apoptosis of Hep3B human hepatocellular carcinoma cells (7). These authors suggested that Ssf-1 might be a negative regulator of apoptosis. In this report, we have shown that Ssf-1 messenger is also regulated during the differentiation of HL-60 cells, a cell line derived from a patient with human promyelocytic leukemia. These cells are induced to differentiate into neutrophil-like

cells by various agents, including G-CSF (12) and dBcAMP (13). Both agents induced a rapid and transient upregulation of Ssf-1 mRNA in HL-60 cells, suggesting that Ssf-1 might play a role in neutrophil maturation. The potential role of Ssf-1 in apoptosis and cell differentiation deserves additional studies.

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