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## SHORT REPORT

**Isolation and characterization of the mouse homolog of *SYT*, a gene implicated in the development of human synovial sarcomas**DRH de Bruijn<sup>1</sup>, E Baats<sup>1</sup>, U Zechner<sup>2</sup>, B de Leeuw<sup>1</sup>, M Balemans<sup>1</sup>, D Olde Weghuis<sup>1</sup>, U Hirning-Folz<sup>2</sup> and A Geurts van Kessel<sup>1</sup><sup>1</sup>Department of Human Genetics, University Hospital Nijmegen, The Netherlands; <sup>2</sup>Department of Medical Genetics, University of Ulm, Germany

In a previous study we reported the isolation of the human synovial sarcoma-associated t(X;18) breakpoint. As a result of this translocation, the *SYT* gene on chromosome 18 fuses to either the *SSX1* or the *SSX2* gene on the X chromosome, depending on the exact location of the breakpoint within band Xp11.2. As yet, little is known about the modes of action of the *SYT* and *SSX* genes and their respective (fusion) products. Here we report the isolation of the mouse homolog of *SYT*, its full length cDNA sequence, its chromosomal localization, and its spatio-temporal expression patterns in adult and embryonic tissues. The *SYT* gene was found to be well conserved during evolution and is part of a region of synteny between the human and mouse chromosomes 18. In early embryogenesis, *Syt* is ubiquitously expressed. In later stages, the expression becomes confined to cartilage tissues, specific neuronal cells and some epithelial derived tissues. In mature testis, expression was specifically observed in primary spermatocytes.

**Keywords:** synovial sarcoma; mouse *Syt* gene/SH2-; SH3-binding domains; annexin-like repeats; chromosome 18

Synovial sarcoma is a soft tissue tumor mainly occurring in the extremities of children and young adults. At the cytogenetic level a characteristic t(X;18)(p11.2;q11.2) has been observed, sometimes as the sole cytogenetic anomaly. It has been assumed that this chromosomal anomaly represents the key molecular event in the pathogenesis of these tumors (Turc-Carel *et al.*, 1987). We and others found that in different t(X;18)-bearing tumors the breakpoints may occur at two distinct sites within band Xp11.2 (de Leeuw *et al.*, 1993b; Shipley *et al.*, 1994). Interestingly, we noted that the occurrence of these different breakpoints correlates with the histologic phenotype displayed by the tumors, i.e., biphasic vs monophasic, respectively (de Leeuw *et al.*, 1993b; Olde Weghuis *et al.*, 1994; Janz *et al.*, 1995). Recently, the t(X;18) chromosomal breakpoint could be isolated (de Leeuw *et al.*, 1993a; 1994a,b). Subsequently, a chimaeric (X;18) cDNA clone was isolated (Clark *et al.*, 1994); and the contributing genes were referred to as *SYT* (chromosome 18) and *SSX* (X chromosome). In

accordance with the observed differences in X chromosomal breakpoints, two alternative fusion genes, *SYT-SSX1* and *SYT-SSX2*, were defined (de Leeuw *et al.*, 1995; Crew *et al.*, 1995). In the *SSX* gene products, Krüppel-associated boxes (KRABs) could be identified (Crew *et al.*, 1995). KRABs are often found in transcriptional regulators which resemble the *Drosophila* Krüppel gene product (Licht *et al.*, 1993; Witzgal *et al.*, 1994). The amino acid sequence of the human *SYT* gene is rich in glutamine, proline and glycine (16.2%, 16.2% and 14.7%, respectively). Within the C-terminal part three putative Src Homology (SH) binding domains could be distinguished (Clark *et al.*, 1994). The presence of these putative SH binding domains suggests that *SYT* is involved in cellular signal transduction pathways (Schlessinger, 1994).

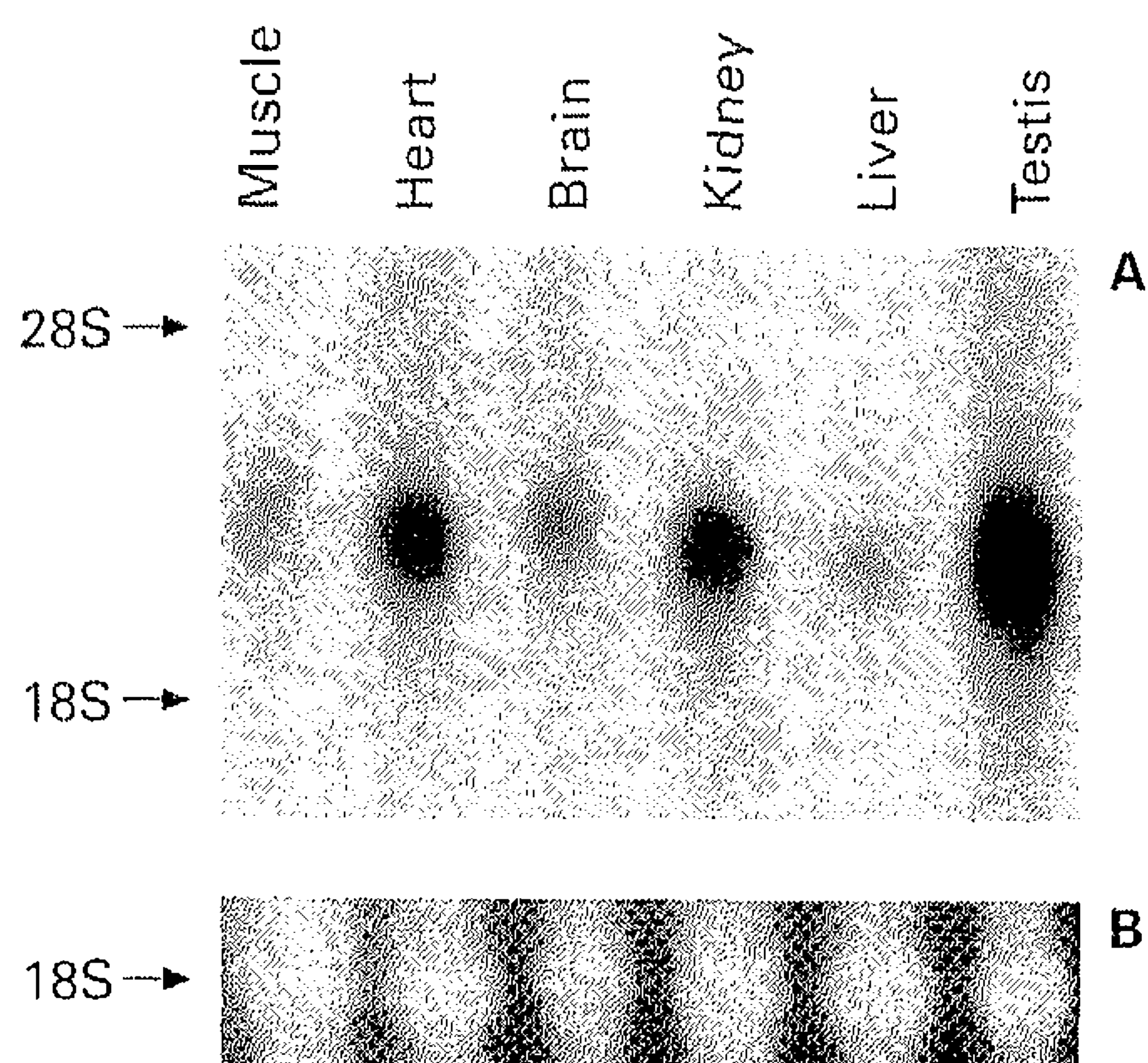
Based on the assumption that conservation between the mouse homolog and human *SYT* would be maximal within the open reading frame, two human cDNA-derived primers (*SYT-UP* 5'-GAAACAT-GTCTGTGGCGTTC-3' and *SYT-REV* 5'-TG GTAAGCGTCTTGCTGTTG-3'; de Leeuw *et al.*, 1995) were selected and applied to a low stringency reverse transcription polymerase chain reaction (RT-PCR) on RNA extracted from mouse fetal brain tissue. Several fragments could be amplified, one of which was used as a probe to screen a mouse fetal brain cDNA library in lambda ZAP (Stratagene), applying standard procedures as described before (de Leeuw *et al.*, 1993b; de Bruijn *et al.*, 1994). One positive clone, with an insert of approximately 3.1 Kilobases (kb), appeared to contain a full length mouse *Syt* cDNA. This fragment was subcloned and completely sequenced (Figure 1). The total length of this mouse *Syt* cDNA clone exceeds the one published for human *SYT* (Clark *et al.*, 1994), which can be attributed to a few distinct differences. Firstly, both the 5' and the 3' untranslated regions of mouse *Syt* are longer than those of human *SYT* and, secondly, in the open reading frame from position 1059 (in the mouse sequence; Figure 1) an addition of 93 basepairs is found. This insertion does not disturb the open reading frame nor does it have any overt effect on the overall amino acid constitution of the predicted mouse *Syt* protein. The sequence of this insert suggests that it is an extra exon spliced into this cDNA, since the ends resemble exon donor and acceptor sites. The homology between mouse and human sequences was found to be high, 90% identity at the DNA level in the open reading frame and 95% identity at the amino acid level, excluding the aforementioned insert. Comparison

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CCTTGCTGGG AGCTGGGGCT CAGCGTAAAG GCCAAGCCGG CCAGCGAGGG ACGCGGCCCG GGAGCATCCT CCCCCACCG CGCGCCCTAA GGTGGAAC TG 160
CCCGGAGGCG GGCCTGGGGC CCCCAGCTCC GCGGGCCCTG GAGCGCTCGG GACTTCGCTGA TCGCGGGCTC GGCGGAAACA TGTCTGTGGC GTTCGCAGCC 200
                                                                                                     M S V A F A A
CCGAGGCAGC GGGCCAAGGG CGAAATCAAG CCGCGCCGCA TCCAGAAGAT CCTGGATGAA AACAACCATC TTATTCAGTG TATAATGGAC TATCAGAACA 300
P R Q R G K G E I T P A A I Q K M L D E N N H L I Q C I M D Y Q N
AAGGGAAGGC CTCGGAGTGC TCGCAGTATC AGCAGATATT GCATACAAAC CTGGTATACC TTGCTACAAAT AGCAGACTCT AATCAAAATA TGCAGTCTCT 400
K G K A S E C S Q Y Q Q I L H T N L V Y L A T I A D S N Q N M Q S L
CTTACCAGCA CCGCCACAC AGACTATGCC AATGGGTCTT GGAGGGATGA GTCAGAGTGG CCTCCACCC CCTCCCGCT CTCACAACAT GCCTTCAGAT 500
L P A P P T Q T M P M G P G G M S Q S G P P P P P R S H N M P S D
GGAAATGGTGC GTGGGGGCC TCCTGCACCA CACATGCAGA ACCAGATGAA CGGCCAGATG CCTGGGCCTA ACCATATGCC AATGCAGGGA CCTGCACCCA 600
G M V G G G P P A P H M Q N Q M N G Q M P G P N H M P M Q G P G P
GTCAGCTCAG CATGACAAAC AGCTCCATGA ATATGCCITC AAGTAACCAT GGCTCCATGG GAGGTTACAA CCATTCCTGTG CCGTCATCCC AGAGCATGCC 700
S Q L S M T N S S M N M P S S N H G S M G G Y N H S V P S S Q S M P
CGTGCAGAAC CAGATGACAA TGAGTCAGGG GCAGCCAATG GGAACACTATG CTCCAGACC AAACA'PGANT ATGCAACCAA ATCAAGGGCC GATGATGCAC 800
V Q N Q M T M S Q G Q P M G N Y G P R P N M N M Q P N Q G P M M H
CAGCAGCCTC CTTCTCAGCA GTACAATATG CCACCTGGAG GGGCACAGCA TTACCAAGGA CAGCAGGCGC CCATGGGGCT GATGGGCCAA GTTAACCAAG 900
Q Q P P S Q Q Y N M P P G G A Q H Y Q G Q Q A P M G L M G Q V N Q
GCAGTCACAT GATGGGCCAG CGACAGATGC CTCCTACAG ACCTCCGCAA CAGGGCCAC CACAGCAGTA CTCAGGCCAG GAAGACTATT ATGGGGACCA 1000
G S H M M G Q R Q M P P Y R P P Q Q G P P Q Q Y S G Q E D Y Y G D Q
ATACAGTCAT GGTGGACAAG GTCCTCCAGA AGGCATGAAC CAGCAATAIT ACCCTGATGG TCATAATGAT TACGGT'ATC AGCAACCGTC G'AT'PCTGAA 1100
Y S H G G Q G P P E G M N Q Q Y Y P D G H N D Y G Y Q Q P S Y P E
CAAGGCTACG ATAGGCCTTA TGAGGATTCC TCACAACAT' ACTACGAAGG AGGAAACTCC CAGTATGGCC AACAGCAAGA CGCTTACCAG GGACCACCTC 1200
Q G Y D R P Y E D S S Q H Y Y E G G N S Q Y G Q Q Q D A Y Q G P P
CACAGCAAGG ATACCCACCC CAGCAGCAGC AGTACCCGGG ACAGCAGGGA TACCCAGGGC AGCAGCAGAG CTATGGTCTT TCGCAGGGCG GTCCAGGTCC 1300
P Q Q G Y P P Q Q Q Q Y P G Q Q G Y P G Q Q Q S Y G P S Q G G P G P
TCAGTATCCT AATFATCCTC AGGGTCAAGG TCAACAGTAT GGGGGCTATA GACCAACACA GCCAGGACCA CCCCAGCCAC CCCAGCAGAG GCCTTATGGG 1400
Q Y P N Y P Q G Q G Q Q Y G G Y R P T Q P G P P Q P P Q Q R P Y G
TACGACCAGG GACAGTATGG AATTACCAG CAGTAAAAAT GTCCTTACAT TCCAATAGCC AGTACCTATT AGCAGCCACG TTGTCACAGC ACTGCACCAT 1500
Y D Q G Q Y G N Y Q Q *
GGACACCCCC CTGGGAAGAC TCCTTCCATF CCAGCTAGGT TTGTGGGAAA ACCTTTGGCT AACTGGCTGC TTCGACAGCA AGTAGCTGTT ATGGT'FTAGT 1600
TTGTAAAGGC TTCGTAGATA CCGATGCACC TGATTTACAG TTCTTACTCT AGATGGCAAC ATTTGGACAGA AAATGCATTG ACGT'GAGGAG TTTGCAGCGG 1700
TTTCAGAACT GTGCTGCAA TGGACTGACA CAGCCTGAAA GGTGTGAGCA GCTGGGTGTG TGTTCGCGGA GCTTCAGGGG GTTTCATACT TCTCCACCGA 1800
TTATTTTGT' AGGGGAAGGG GGAATGTAC ACTTCTACA GCAGCAATAT TTGGGCTATT ATGTTATTTT CATG'IGATAA ATATGCAAAG CGGTACACTA 1900
CACACTGGGC AGGAATCAGA ACCCTGT'AA TGTGGAGTGT GGTAGATGCT CCGT'GCTGTG GTGCTCTGAA GACAGGCGAG GGGAGGCAGA ACCCACCACA 2000
GGCCCGCTGT TAGT'CTT'AG AGGAAACTCC TCCTCTCTCT ATCTACCAGA TTAGCAAGGG CGCTGTGATA CAGT'TTTTTG AGTACAAAGA CAT'TTTTTAA 2100
AAAGCCTTCC AGTTTGTGC AT'AAAACCT TTTTGTAAAT ATGGTTTATA ATACTGTTT CAAACGCAAG GCAATAATTA TGTTCATCT GTGAAC'TTG 2200
GCAGGTTTGT GTAAAAGGAG GGAAGCCTCT CTTAAAACAG CAATAACAGA AAAGGAGGAA GCGGGATGTT TTTACC'TTGT CTTGTAATCA GGGAGCTCTC 2300
ACCACGT'AG AGAGGAGGCA GCATTTGGTCT CACCTTACTG TTTT'ACAT TACCATGATT GGTTCATGGA GCAGGGAGGA GTCCACGAGA CTTACACAGC 2400
TTGTGCTTTA ACTTCTTAA CTGGGCACAA GCAAAGGGCG CCT'CGTGT' CCTCTCTTCA TCTTAGTTAA TCGCGGAGGA AAATGCTTTG ATGGCCATTT 2500
CTCATTCGCA CTGAAAGCCG AGAGGTGACA TTTTACGGTT TC'TGT'TTT AAGCACGACA TACTTAATCA TTATTTGAGA CTGATTATTT TAGCTAAAAT 2600
TCGGGATATG CCATGGGGCA AGAAAACATG TACTGAGAGA TTTCTAAACA CATCTATTTA AGCATACTTT AAAAATATCT AGCCCAAAGG TAAGTT'GCTG 2700
TATCTCACA GTTGTCTGCA TCCAGGCAAT ATGACTGAAT ATAACATATC TTTGTAATTT AAT'TAGTTT TGCCTACTCT AACTGAAAAC AGAACAGAAG 2800
GACTGCCATA AATCCAAAGA AGCAAAGTGT ACTGTTGTC ACA'FACTGTA ATCAGAGGAG GGGTTCAAT GTGCTTGGAT GAGAGTGTGT GTGTTTAAAG 2900
TCAGAGTATA GGGTGTCTT CAACTTGGAC AGTAAAAAT AGGCATCAAG TGTGAACCGG TGAGGCGTGG ACAGCCTTCT TGTGACTGAG ATGCTTGTAA 3000
GTTCGTGTC AGG'PCTCCA CCACTGTGTA CTTTATTGCT ATTTAAAAC GTATCAACTC TAACGAAAAGA ATAAAT'ATT TGTGATTTTA AAAAAAAAAA 3100
AAAAAAAA 3107

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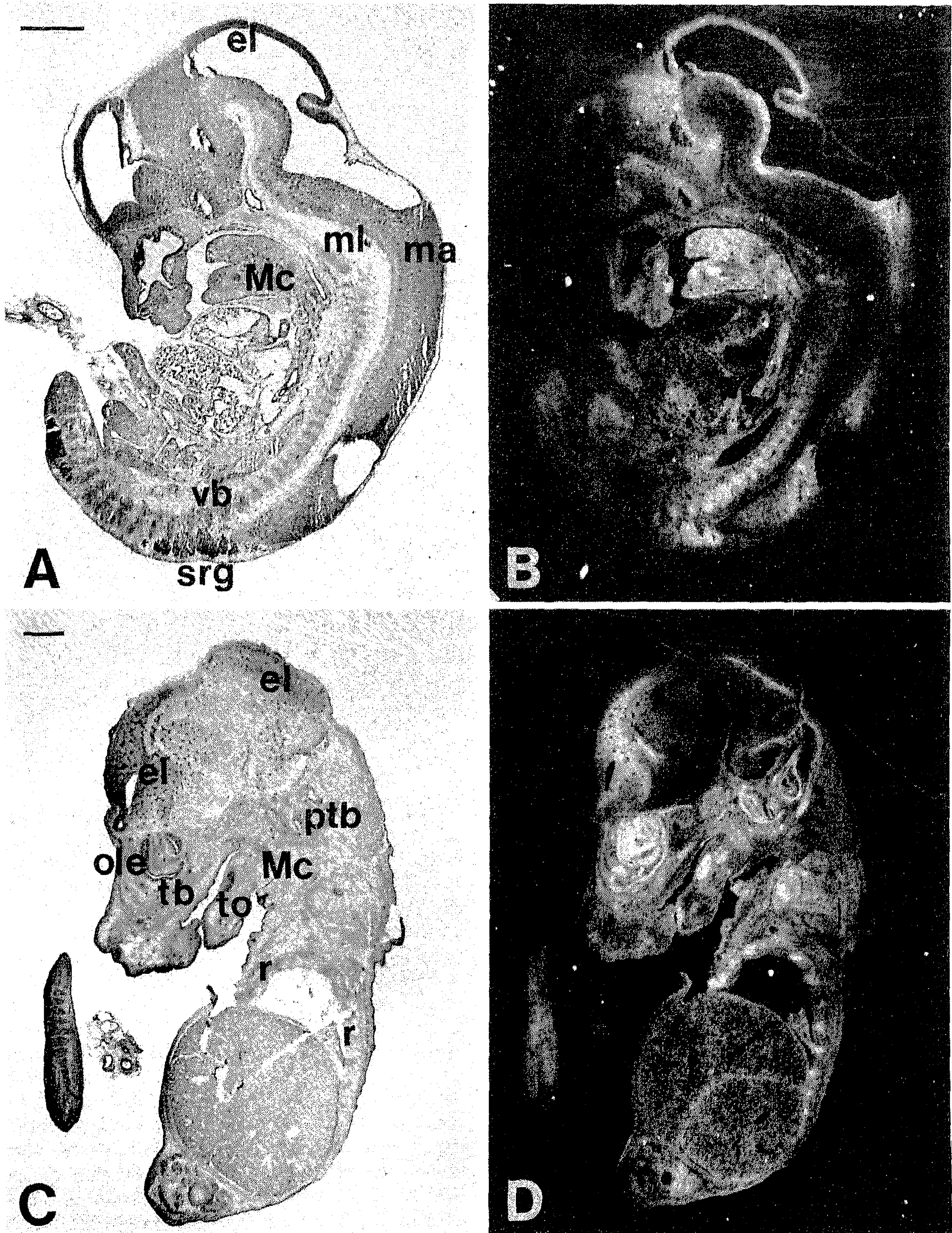
**Figure 1** Nucleotide sequence of the mouse *Syr* cDNA and the derived amino acid sequence with start- and stopcodons printed in bold. Two putative poly-adenylation signals at positions 3041 and 3070 are also printed in bold. The arrows indicate two imperfect direct repeats, which resemble repeats found in annexin-like proteins. Two putative SH2- and one putative SH3-binding domain starting at amino acids 376, 413 and 392, respectively, are underlined. Nucleotides were analysed on an automated DNA sequencer (ABI 373A) using the Taq Dye Deoxy Terminator Cycle Sequencing kit (Applied Biosystems). The sequence accession number for this cDNA is X93357



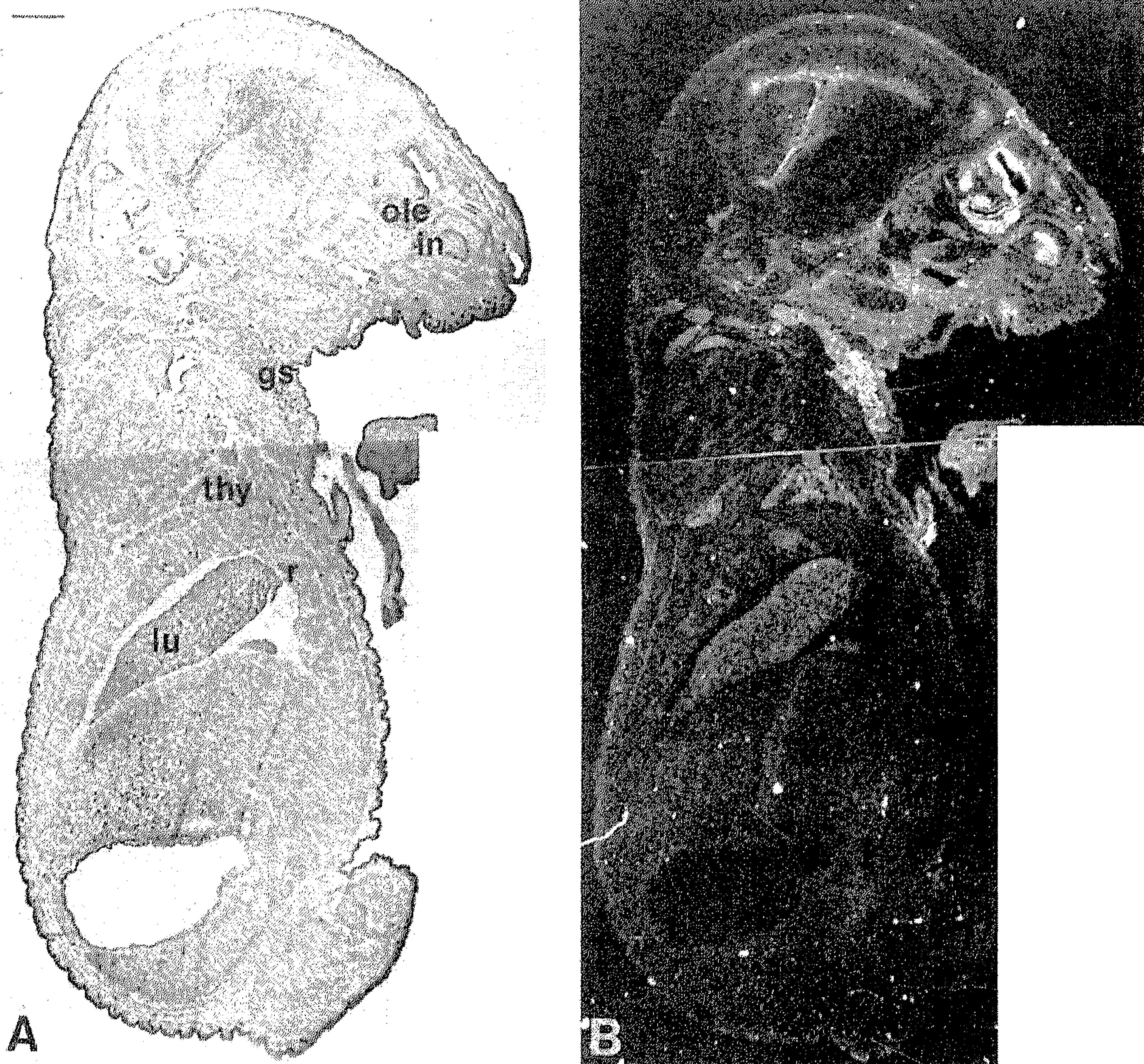
**Figure 2** Multiple tissue Northern blot, hybridized with the mouse *Syr* cDNA (a). Stronger signals can be observed in the lanes containing heart, kidney and testis RNA. As an indication for the total amount of RNA loaded, (b) shows the corresponding ribosomal 18S band, stained with Ethidium Bromide. Probe labeling and hybridization procedures were carried out as described before (de Bruijn et al., 1994)

of the human and mouse cDNA sequences shows that the start codon suggested for the human *SYT* gene (Clark *et al.*, 1994) exactly coincides with the first ATG present in mouse *Syt* at position 179 (Figure 1). Further confirmation that this is the actual translation

initiation site comes from the observation that the homology between mouse and human *Syt* cDNA drops to about 70% before this ATG. Computer analysis of the total mouse cDNA insert using the TESTCODE program shows that triplets in the open reading frame



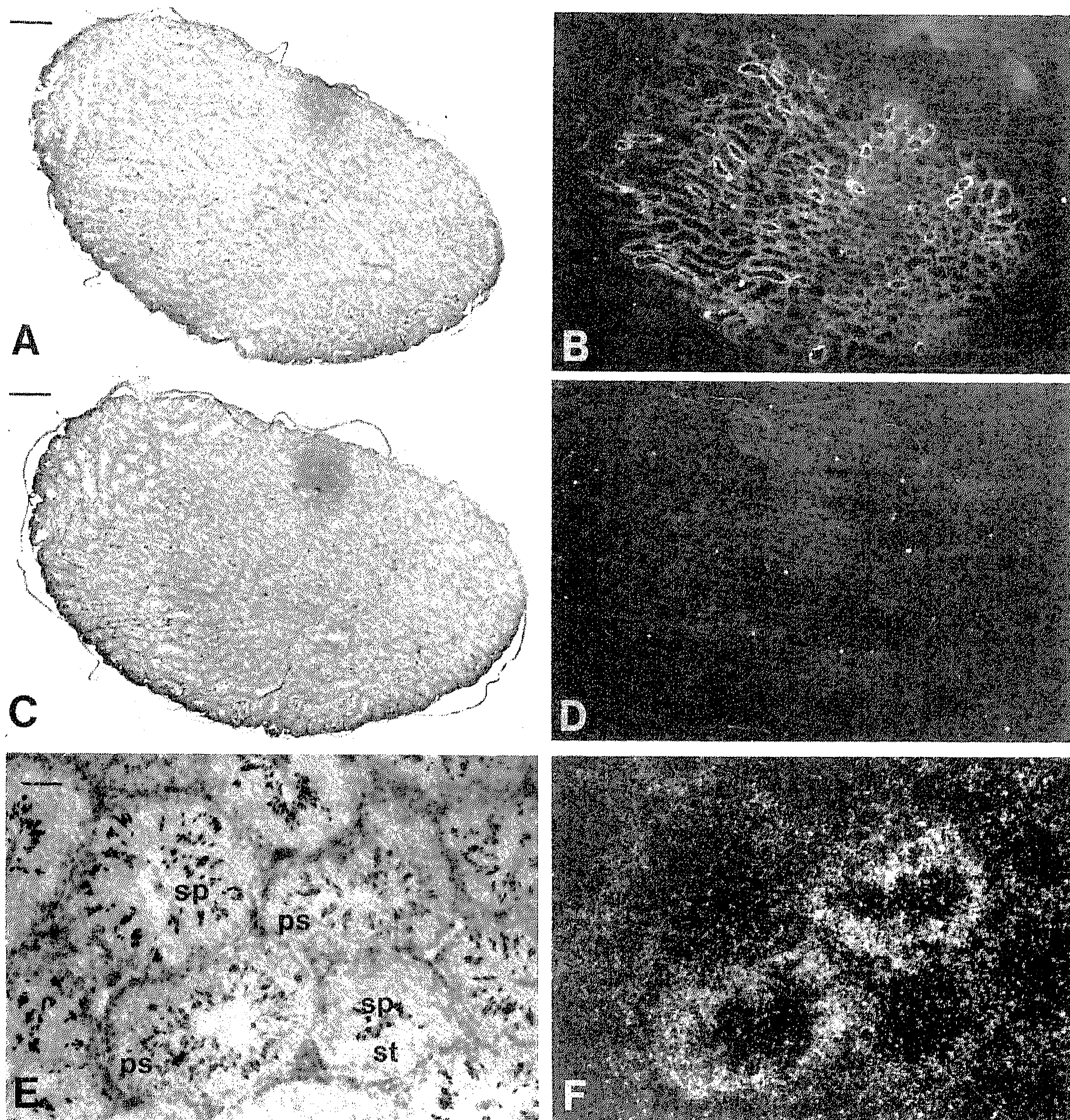
**Figure 3** Sagittal sections through a day 12.5 p.c. (a and b) and a day 14.5 p.c. (c and d) mouse embryo hybridized with a *Syt* RNA probe. Left brightfield and right darkfield illumination. At 12.5 d.p.c. expression can be observed in Meckel's cartilage (Mc), vertebral bodies (vb), spinal root ganglia (sgr), the endymal lining of the ventricles (el) and the mantle layer of the central nervous system (ma), with clear absence of signal in the marginal layer (ml). At 14.5 d.p.c. expression can be observed in the ribs (r), olfactory epithelium (ole), turbinate bone (tb), tooth bud (to) and the petrous part of the temporal bone (ptb). Expression continues in Meckel's cartilage and the endymal lining of the ventricles. Bar = 1 mm



**Figure 4** Parasagittal section through a day 18.5 p.c. mouse embryo. Expression has become more restricted. Pronounced expression can be observed in the olfactory epithelium (ole), incisor (in), glandula submandibularis (gs), thymus (thy) and lung (lu). Expression in the ribs (r) has disappeared. Bar = 1 mm

have a non-random composition of every third base (data not shown). This non-randomness can be attributed to the presence of an expressed sequence (Fickett *et al.*, 1982). Analysis of the amino acid sequence shows that many of the features described for human *Syt* can also be found in the mouse homolog. Searches of different databases using the FASTA and BLAST search algorithms did not yield strong homologies to any other known gene. Mouse *Syt* is rich in glutamine, proline and glycine (18.6%, 15.8% and 13.8%, respectively). Glutamine/proline rich stretches have previously been found to be present in transcriptional activators. Interestingly, within the mouse *Syt* glutamine/proline rich stretch, we found an imperfect direct repeat of 13 amino acids (Figure 1, positions 344–356 and 357–369). Repeat units, reminiscent of this one have also been found in Calcium binding proteins (*Annexin VII*, Greenwood *et al.*, 1991) and cyclic AMP binding proteins (*CABP*, Grant *et al.*, 1990) of *Dictyostelium discoideum*. These repeat units may vary in length and number of repeats, but they are also invariably rich in tyrosine residues. It has been suggested that these structures form beta sheets which may be involved in protein-protein interactions (Zhang-Keck *et al.*, 1993).

The total mouse *Syt* cDNA clone was used as a probe on Northern blots containing total RNAs extracted from adult mouse muscle, heart, brain, kidney, liver and testis (Figure 2). Overall, ubiquitous expression was observed with relatively higher mRNA levels in heart, kidney and testis. In order to extend these analyses and to reveal spatio-temporal expression patterns during mouse embryogenesis, mRNA *in situ* hybridizations were carried out as described before (Schmidt *et al.*, 1989) on serial sections obtained from mouse embryos at different stages of development using *Syt* 'anti-sense' RNA as a probe. Figure 3b shows the expression pattern in a 12.5 day post coitum (d.p.c.) mouse embryo. At this developmental stage, again *Syt* appears to be ubiquitously expressed. Relatively strong expression is detected in cartilage and specific neuronal cells. Expression in cartilage is evident in the primordia of the vertebral bodies and in Meckels' cartilage. In neuronal cells, elevated expression marks the ependymal lining of the ventricles, the spinal root ganglia, and – with less intensity – the mantle layer of the spinal cord with apparent absence of signals in the adjacent marginal layer. At stage 14.5 d.p.c. the pattern of expression is more confined. In Figure 3d, a more parasagittal section is shown.



**Figure 5** Sections through a testis taken from an 80 day old adult mouse hybridized with a *Syt* RNA probe. Left brightfield and right darkfield illumination. Control hybridizations were performed with a sense riboprobe (c and d). (e and f) show higher magnification images of two positively hybridizing tubuli seminiferi from b. *Syt* expression can be observed in a broad band of primary spermatocytes (ps). Other, non-hybridizing, cell types are mature sperm (sp) and spermatids (st). Bar = 300  $\mu$ m in a-d and 30  $\mu$ m in e-f

Highest expression is seen in cartilage primordia of the ribs, of the petrous part of the temporal bone, which surrounds the stato-acoustic organ, and of the turbinate part of the maxilla, which surrounds the olfactory apparatus. Prominent expression is also seen in Meckel's cartilage and in the tooth bud of the first molar. In neuronal tissues, the expression is reduced as compared to stage 12.5 d.p.c. but is evident in the ependymal cells of the central ventricles and in the neurosensory olfactory epithelium. In further sections, enhanced expression was noted in both cell layers of the retina, kidney and lung (not shown). At stage 18.5 d.p.c. when ossification has replaced chondrification in most bones, *Syt* expression is reduced to background levels in bones (Figure 4b). This is most evident in the olfactory apparatus where a prominent signal is present only in the sensory epithelium. At this stage, high expression is observed in the primordia of the upper

incisor, the glandula submandibularis and, somewhat less, in the thymus. Compared to earlier stages, the expression in lung and kidney is reduced.

In accordance with the abovementioned Northern blot analyses, higher levels of expression were observed in adult mouse testis and kidney. In kidney, an overall elevated level without any specification to certain structures was observed (not shown). In the testis of an 80 days old mouse, expression is prominent in certain tubuli seminiferi (Figure 5b). At enhanced magnification it is evident that expression is found only in primary spermatocytes which pass through the late prophase stages of the first meiotic division. In those tubuli with an elevated level of expression, a higher proportion of cells is represented by primary spermatocytes. According to the spermiogenic stages as defined by Oakberg (1956) expression is highest in tubuli from stage IX to XI. During these stages, a

broad layer of primary spermatocytes passes from pachytene to diplotene. *Syt* expression appears to be confined to these cells (Figure 5f).

To elucidate the genomic structure and to facilitate the chromosomal localization of the mouse *Syt* gene, we screened a mouse genomic library in EMBL3 using the total mouse *Syt* cDNA as a probe. Several overlapping phages were recovered and a preliminary intron-exon map of the gene was constructed. Partial sequence analysis of these phages confirmed the actual presence of the mouse *Syt* gene. The chromosomal localization of the gene was determined by fluorescence *in situ* hybridization (FISH) using one of the *Syt*-positive phages as a probe, essentially as described by de Leeuw *et al.* (1993a). In total, 30 mouse metaphase spreads were analysed and, in all cases, positive signals were observed on chromosome 18, region B1, mostly on both homologs (not shown). No specific signal was observed on any of the other mouse chromosomes. This observation is in full agreement with previous findings indicating that the mouse genomic region harboring the *Syt* gene (18B1) is syntenic with human chromosome 18, region q11-q12, also harboring the *SYT* gene (Overhauser *et al.*, 1995).

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In conclusion, the mouse homolog of human *SYT* appears to have been well conserved over the course of evolution, both in chromosomal location and in constitution. We have shown that the expression of mouse *Syt* is restricted to cartilage forming cells, specific neuronal cells and some epithelial derived tissues. A highly specific expression is observed in mature testis. Particularly interesting is the notion that several parts of the *Syt* protein may be implicated in protein-protein interactions, either via SH2/SH3-binding domains or via annexin-like domains.

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