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Research article

Genomic organization and the tissue distribution of alternatively spliced isoforms of the mouse *Spatial* gene

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Abstract

Background: The stromal component of the thymic microenvironment is critical for T lymphocyte generation. Thymocyte differentiation involves a cascade of coordinated stromal genes controlling thymocyte survival, lineage commitment and selection. The "Stromal Protein Associated with Thymii And Lymph-node" (*Spatial*) gene encodes a putative transcription factor which may be involved in T-cell development. In the testis, the *Spatial* gene is also expressed by round spermatids during spermatogenesis.

Results: The *Spatial* gene maps to the B3-B4 region of murine chromosome 10 corresponding to the human syntenic region 10q22.1. The mouse *Spatial* genomic DNA is organised into 10 exons and is alternatively spliced to generate two short isoforms (*Spatial*-α and -γ) and two other long isoforms (*Spatial*-δ and -ε) comprising 5 additional exons on the 3' site. Here, we report the cloning of a new short isoform, *Spatial*-β, which differs from other isoforms by an additional alternative exon of 69 bases. This new exon encodes an interesting proline-rich signature that could confer to the 34 kDa Spatial-β protein a particular function. By quantitative TaqMan RT-PCR, we have shown that the short isoforms are highly expressed in the thymus while the long isoforms are highly expressed in the testis. We further examined the inter-species conservation of *Spatial* between several mammals and identified that the protein which is rich in proline and positive amino acids, is highly conserved.

Conclusions: The *Spatial* gene generates at least five alternative spliced variants: three short isoforms (*Spatial*-α, -β and -γ) highly expressed in the thymus and two long isoforms (*Spatial*-δ and -ε) highly expressed in the testis. These alternative spliced variants could have a tissue specific function.

Background

The immune system is composed of many interdependent cell types that collectively protect the body. The hematopoietic precursors leave the bone marrow and migrate into the thymus, primary site of T cells development, providing a unique microenvironment that efficiently

generates functional T lymphocytes. This maturation requires the interaction of immature thymocytes with the thymic stroma. The lympho-epithelial interaction delivers signals necessary for survival, proliferation, differentiation and selection of developing thymocytes [1]. After a remarkable maturation process, mature T cells are then released into the bloodstream and migrate to secondary lymphoid organs, such as the spleen and lymph nodes.

Although the T cell differentiation process is well documented, little is known about the underlying stromal molecules involved in the lympho-epithelial interaction. In order to identify new stromal genes potentially involved in these mechanisms, we developed a systematic approach of gene expression profiling to evaluate, in comparison to the *wild-type* littermate, the gene expression of several thymus deficient mice showing a blockage at different stages of T cell development [2-4]. Among the identified stromal genes, we selected a gene, dependent on the three-dimensional organization of the thymus, named *Spatial* for "Stromal Protein Associated with Thymii And Lymph-node" [5]. RT-PCR analysis on 48 distinct mouse tissues showed that *Spatial* is highly expressed in the thymus and testis. In the testis, *in situ* hybridization experiments showed that *Spatial* expression is tightly regulated and restricted to step 2–10 in haploid round spermatids during spermiogenesis [6].

Here, we describe the genomic organization of the *Spatial* gene and subsequently identify a new alternatively spliced isoform expressed in the thymus. In addition, we precisely evaluate the tissue distribution of the alternative spliced variants of *Spatial* gene by quantitative TaqMan RT-PCR.

Results and discussion
Chromosomal localization
We determined the chromosomal localization of *Spatial* gene by fluorescence *in situ* hybridization using a 40 kb cosmid isolated from the rzpd genomic library. 98% of 30 analysed metaphase cells showed specific fluorescent spots on the B3-B4 region of murine chromosome 10 (Fig. 1A). This single signal strongly suggests that *Spatial* does not belong to a gene family. This region contains the *Sim1* [7], *Zfa* [8], *Hsf2* [9] and *Edar* [10] genes in the B3 band and *Eif4ebp2* [11], *Sgpl1* [12], *Ddx21* [13] and *Gp49b* [14] genes in the B4 band (Fig. 1B). Furthermore, the human synthenic B3-B4 region is divided into two regions located on chromosome 6 and 10 where *SIM1* and *HSF2* genes are located on chromosome 6 and the *SGPL1*, *DDX21* and *EIF4ebp2* genes were mapped on chromosome 10. This observation suggests that these genes are respectively located above and below the mouse translocation points T(2; 10) and T(10; 18). In parallel, by BLAST analysis, we identified a chromosome 10q22.1 human contig which displays similarities to *Spatial* gene (Genbank Accession

No NT_008583) where no pathologies were described in this region until now. All together, these data show that *Spatial* is located on the mouse chromosome 10-B4 band between *Ddx21* and *Eif4ebp2* (Fig. 1C).

Identification of a new alternative spliced variant of Spatial
In order to study the stromal molecular mechanisms involved in T cell development, we isolated new genes, by quantitative differential screening using array technology, between *wild-type* and several *knock-out* mice models displaying a stromal disorganization. We screened from an adult mouse thymus library (<u>M</u>ouse <u>T</u>hymus <u>A</u>dult, MTA) three *Spatial* cDNA clones: MTA.H09.096, MTA.C03.094 and MTA.F04.072 (GenBank Accession No. W91576, W91584, AY243458 respectively). In addition, by screening a 15 days embryonic thymus mouse library (<u>M</u>ouse <u>F</u>etal <u>T</u>hymus, MFT, library accessible in the RZPD resource center), another Spatial cDNA clone was isolated: MFT.G17.012 (GenBank Accession No. AY243459).

The sequencing of these clones showed that MTA.C03.094 is similar to MTA.F04.072 while MTA.H09.096 and MTA.F04.072 differ by a 102 bases additional exon, corresponding to two adult variants already described in the thymus (GenBank Accession No. AF257502 and AF257503) [5]. In addition, sequence analysis of the MFT.G17.012 showed that it contains an additional exon of 69 bases. We confirmed the presence of this new exon by performing RT-PCR analysis on total RNA extract from adult mouse thymus, using specific primers for this new additional exon (Fig. 2A). Thereafter, we cloned and fully sequenced this third isoform reporting the expression of this new *Spatial* splicing variant in the thymus (GenBank Accession No. AY243457). According to the size of alternative spliced variants, the longest and the shortest isoforms were respectively named *Spatial*-α (1035 bp) and *Spatial*-γ (933 bp) and the third new mid-sized isoform *Spatial*-β (1002 bp) (Fig. 2A). In addition, we already described two other isoforms in the testis differing by the same 102 bases alternative exon mentioned above: *Spatial*-ε (1454 bp) for the longest (GenBank Accession No. AF521592) and *Spatial*-δ (1353 bp) for the shortest (GenBank Accession No. AF521591) [6]. In order to study the protein product of Spatial, we have developed a polyclonal antibody to be able to recognize all isoforms. As shown in figure 2B, this antibody reacts with the three protein products of thymic isoforms on the nuclear extract. The apparent molecular masses of the upper and the lowest bands correspond to Spatial-α and Spatial-γ at 38 and 32 kDa respectively [5]. In the cytosolic fraction, we can detect Spatial-α which is probably not yet translocated into the nucleus. On the basis of the size of Spatial isoforms, we have deduced that the third band at 34 kDa probably corresponds to the newly described Spatial-β
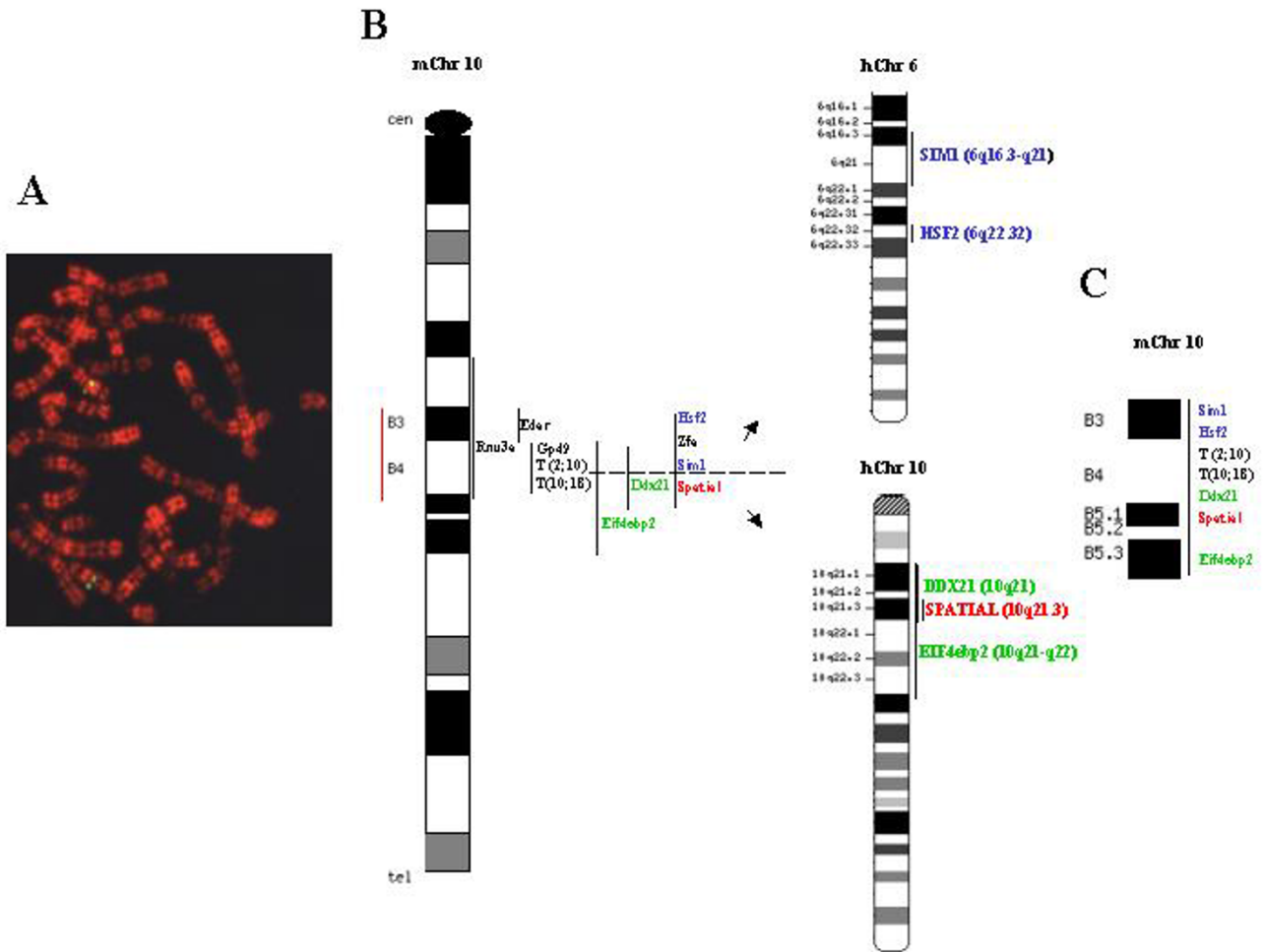
Figure 1
Chromosomal localization. (A) Localization of the *Spatial* probe on WMP murine metaphase: R-banded chromosomes are red-stained with propidium iodide. The FITC fluorescent signals (green), corresponding to *Spatial* gene, are located on chromosome 10 and belong to the robertsonian translocation Rb(10; 17). **(B)** On the left, the map of mouse chromosome 10 zooming on B3-B4 bands where are localized 2 translocation points, and on the right, 2 human syntenic regions located on Ch6 and Ch10. **(C)** The repartition of genes within B3 to B5.3 bands on mouse chromosome 10.

suggesting that this isoform would generate a protein in the thymus.

Organization of Spatial alternative spliced variants
We determined by analyzing the mouse genomic contig (*Mus musculus*, Genbank Accession No. NT_039495) that *Spatial* is composed of 10 exons (Fig. 3A). Four exons of *Spatial* are present in *Spatial*-α, -β, -γ isoforms (exons 2, 3, 4 and 5) and 9 exons in *Spatial*-δ, -ε isoforms (exons 1, 3, 4, 5, 6, 7, 8, 9 and 10) (Fig. 3B). *Spatial*-α and *Spatial*-ε contain an alternatively spliced internal region of 102 bases located in exon 4 and the new described *Spatial*-β contains an alternatively spliced internal region of 69

bases in exon 5. The exon organization of *Spatial*-δ and -ε shows that 5 exons are added at the 3' end compared to *Spatial*-α, -β, -γ. In addition, the start and the stop codons are respectively localized in exon 2 and exon 5 for *Spatial*-α, -β, -γ while they are localized in exon 1 and exon 10 for *Spatial*-δ, -ε. We classified *Spatial*-α, -β, -γ as short isoforms and *Spatial*-δ, -ε as long isoforms. The exon length and the exact position of the start and the stop of different *Spatial* isoforms were determined by detailed analysis of mouse chromosome 10 (Table 1A,1B) [15]. Furthermore, short isoforms contain in the 3' end of exon 5 a nuclear localization signal (NLS) allowing the transport of the protein from the cytosol to the nucleus. This observation strongly
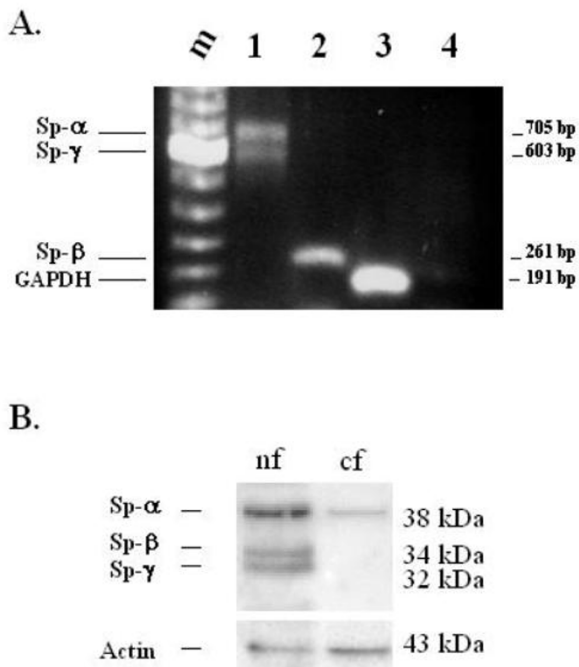
Figure 2
Study of Spatial-β expression in the thymus. (A)
Expression of *Spatial*-β mRNA in the mouse thymus by RT-PCR; the amplicons were the short *Spatial* isoforms: *Spatial*-α, -β, -γ and the housekeeping gene *GAPDH*. The size of the amplicons are indicated. m, 100 bp DNA marker; lane 1, positive control, *Spatial*-α, -γ; lane 2, *Spatial*-β; lane 3, *GAPDH* and lane 4, negative control. For the amplification of *Spatial*-β, we used forward primer specific to the additional 69-bases while the reverse primer was specific to the exon 5. **(B)** Analysis of Spatial-β protein expression in the thymus; in the nuclear fraction (nf), bands at 38, 34 and 32 kDa are detected corresponding to Spatial-α, Spatial-β and Spatial-γ respectively. In the cytosolic fraction (cf), only one band at 38 kDa is detected which corresponds to Spatial-α that could be under nuclear translocation process.

suggests that long and short isoforms could have different subcellular localizations which potentially leads to different functions.

Tissue distribution of mouse Spatial isoforms

Previous RT-PCR analysis on a large panel of mouse tissues showed that *Spatial* is highly expressed in the thymus and testis, but also to a lesser extent in brain cortex, cere-

bellum and hippocampus [6]. In order to precisely decipher the tissue distribution of short and long *Spatial* isoforms, quantitative TaqMan RT-PCR was performed on the thymus, testis as well as the kidney as a negative control. The expression of *Spatial* isoforms in different adult tissues was measured relatively to the expression of the housekeeping 18S-rRNA gene.

This analysis shows a residual expression of 1.36×10^{-4} RQ value in the kidney for the two isoform types confirming a null expression, while short and long *Spatial* isoforms were differentially expressed in the thymus and the testis. Short isoforms are highly expressed in the thymus whereas long isoforms are highly expressed in the testis. Short isoforms are expressed in the thymus 85-fold higher than the testis and an expression of 1.18×10^{-2} RQ value is observed in testis signing a basal expression (Fig. 4A). Long isoforms are expressed in the testis 25-fold higher than the thymus and in this case the basal expression in the thymus is 3.9×10^{-2} RQ value (Fig. 4B).

This study reveals a mirror image of short and long isoforms expression in these organs. Actually, the basal expression detected by RT-PCR experiments which is very sensitive and known to amplifiy very low expression in classical conditions (30 cycles) can lead to an over estimation of the expression level analysis. Indeed, in the thymus only the short isoforms are translated and give rise to three corresponding proteins (see above). In addition, previous analyses on thymus sections have shown that *Spatial* gene is expressed by subcapsular stromal cells which are resident cells known to be involved in thymocytes development [16]. In testis, the *Spatial* gene is expressed in round spermatids which are migrating cells representing a cell stage prior to spermatozoa maturation [17]. In conclusion, short isoforms are highly expressed in the thymus and at a basal-rate in testis whereas long isoforms are highly expressed in the testis with a basal-rate in thymus. Major isoforms type are expressed in specific cell types suggesting a tissue specific function.

Comparison of the mouse Spatial ortholog protein sequences

We further examined the inter-species homologies by performing a BLAST search of the Expressed Sequence Tag (EST) database with *Spatial* mouse cDNA and identified several mammalian ESTs of pig (*Sus scrofa*, GenBank Accession No. BM484264) and bovine (*Bos taurus*, GenBank Accession No. BF077282) in addition to rat (GenBank Accession No. XM_228291) and human (GenBank Accession No. AK057382) [18,19]. The analysis of the rat EST showed that it corresponds to the mouse *Spatial*-ε isoform. In addition, bioinformatic studies of the rat contig (*Rattus norvegicus*, Genbank Accession No NW_043448) showed that the corresponding EST, in
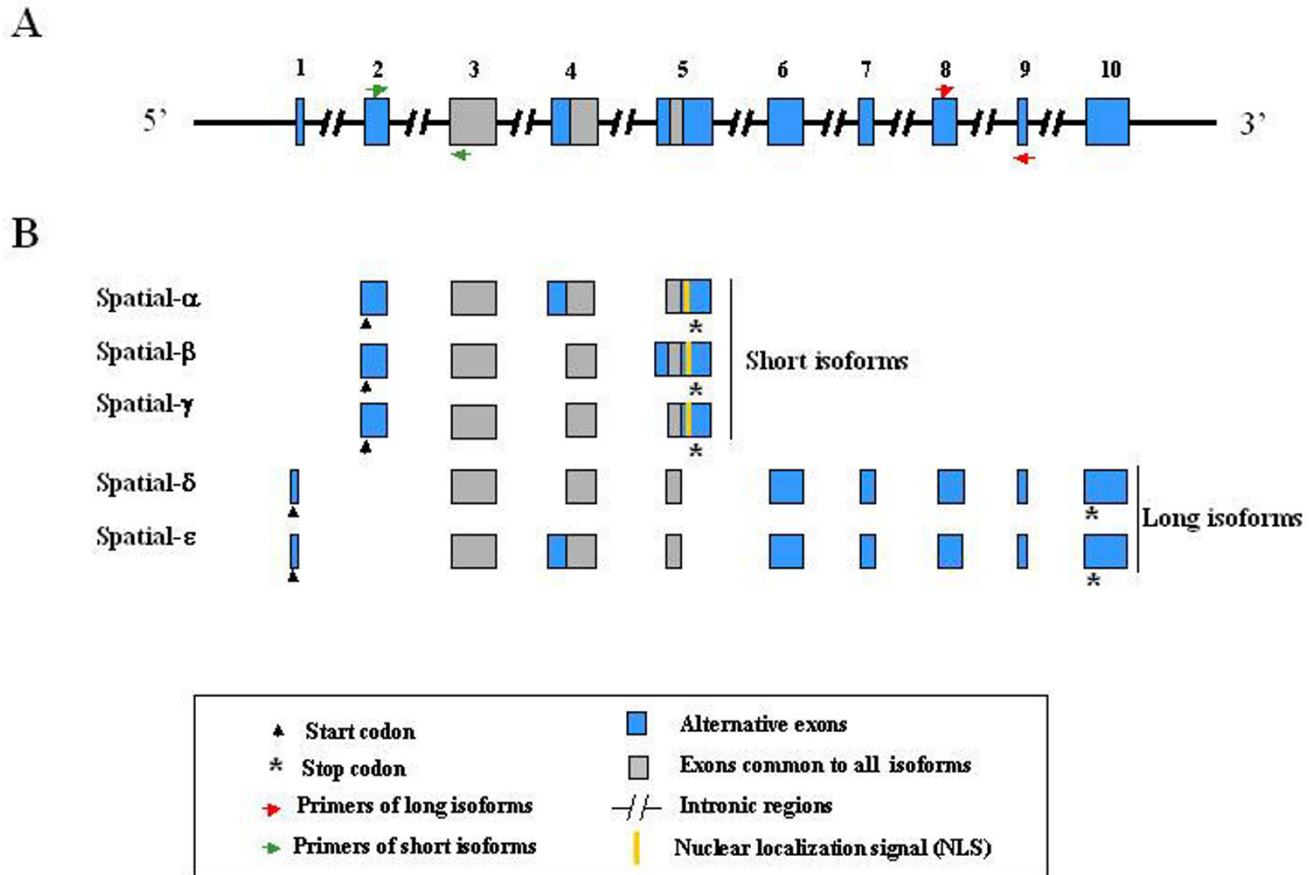
Figure 3
Genomic organization and alternative splicing pattern of *Spatial* gene in the mouse. (A) The mouse genomic sequence corresponding to the *Spatial* gene constituted of 10 exons. Alternative spliced exons are represented by blue boxes and common exons of the different *Spatial* isoforms are represented by gray boxes. Green and red arrows correspond to the designed primers specific for the *Spatial* short and long isoforms respectively used for the quantitative TaqMan RT-PCR experiment. **(B)** mRNA organization of *Spatial* isoforms: the *Spatial* short mRNA isoforms (*Spatial*-α, -β, -γ) differ by a 102 bp additional exon for *Spatial*-α and 69 bp for the newly described *Spatial*-β comparing to the shortest *Spatial*-γ isoform. The NLS encoding sequence is represented by a yellow box, specific for short isoforms. The *Spatial*-δ isoform differs from *Spatial*-ε isoform by a 102 bp additional exon. The predicted start codon indicated by (Δ) and the stop codon indicated by (*) are localised in exon 2 and exon 5 for short isoforms and in exon 1 and exon 10 for long isoforms, respectively. Long and short isoforms differ by the addition of 5 exons in the 5'end of long isoforms.

comparison to the mouse *Spatial*-ε isoform, contains an additional exon in the 5' end separated from the second exon by an intron of about 8 kb. Therefore, we performed an RT-PCR analysis with primers specific for the first predicted exon in the rat and deduced that this exon does not exist (data not shown). This result indicates that the *Spatial*-ε mRNA contains 9 exons in rat as in mouse, while it is composed of 11 exons in human. The alignment of the deduced amino acid sequences is shown in figure 5 [see

Additional file 1]. As a reference, we have chosen the mouse Spatial-α protein, that shows 90% identity with other mouse Spatial isoforms and 55% of inter-species homology. However, we did not find any EST from widely sequenced non-mammalian eukaryotes such as *Drosophila melanogaster*, *Caenorhabditis elegans* and *Saccharomyces cerevisae*.

Table 1: Exon-intron organization of *Spatial* gene in the mouse chromosome 10. (A) The exact size as the position of the start and the end of each exon are precisely calculated for different *Spatial* isoforms. The exon 4 contains two alternatively spliced internal regions (4 a, b) while the exon 5 contains three alternatively spliced internal regions (5 a, b, c). (B) The exact position of the start, the stop and the polyA tail is calculated for short and long isoforms. Feature positions in bp are calculated relatively to position 63050000 of the mouse chromosome 10 (Ensembl build 32). Introns can not be determined because the genomic sequence contains stretches of nucleotides.

A

Exon	Start	End	Length	Short isoforms			Long isoforms	
				Spatial-α	Spatial-β	Spatial-γ	Spatial-δ	Spatial-ε
1	3873	4027	155				3873...4027	3873...4027
2	4834	4975	142	4834...4975	4834...4975	4834...4975		
3	5303	5550	248	5303...5550	5303...5550	5303...5550	5303...5550	5303...5550
4 ab	8090	8350	261	8090...8350				8090...8350
4 b	8192	8350	159		8192...8350	8192...8350	8192...8350	
5 abc	9093	9543	451		9093...9543			
5 bc	9160	9543	384	9160...9543		9160...9543		
5 b	9160	9239	80				9160...9239	9160...9239
6	12958	13149	192				12958...13149	12958...13149
7	14960	15041	82				14960...15041	14960...15041
8	15696	15837	142				15696...15837	15696...15837
9	17083	17135	53				17083...17135	17083...17135
10	18002	18242	241				18002...18242	18002...18242

B

	Start	Stop	PolyA Signal
Short isoforms	4917	9285	9499
Long isoforms	3984	18079	18201

Bioinformatic analyses have predicted that the molecular weights of Spatial-α, -β and -γ are 25.8, 24.6 and 22.2 kDa respectively and present an isoelectric point of 10. The apparent mobility of the *in vivo* proteins (38, 34 and 32 kDa) is greater than the predicted mass based on the cDNA sequence (Fig 2B), this could be explained by a post-translational modifications of these proteins. In addition short isoforms where the NLS sequence is conserved, are detected in the nuclear fraction of the thymus and present proline-rich domains (10%) as there are two other positive amino acid rich domains (arginine, lysine, histidine) which seem to exhibit homology with zinc-finger proteins known to be involved in protein-DNA interaction [20], but the C2H2 consensus motif is not conserved in Spatial proteins. In addition, the 23 amino acid alternative exon (residues 190–212), only found in Spatial-β, presents an interesting proline-rich signature (residues 196–208) that could confer to Spatial-β a particular function. Moreover, these short isoforms possess a secondary structure with 72% of random coil, suggesting

an unstable structure. So, these nuclear proteins may have to interact with DNA and other cofactors in order to stabilise their conformations. Concerning long isoforms, Spatial-δ and -ε are encoded with a 40.2 and 43.8 kDa prediction molecular mass and present an isoelectric point of 6.5. In contrast to short isoforms, they lost the NLS sequence but gained an additional rich leucine sequence (15.5%) in the 3' end, with a high percentage of alpha-helix (44.5%) suggesting a more stable structure.

Taking in consideration all these data, Spatial is a relatively well conserved protein in mammals. Long isoforms observed in human, mouse and rat display alpha-helix in the C-terminal region suggesting a stable structure of the protein. Short isoforms lack any similarity to other known domains apart the putative zinc-finger homology. However, conserving the NLS sequence, short isoforms encode nuclear factors which could play a role in transcriptional regulation cell process. Interestingly, we describe here a new short isoform Spatial-β, which has an additional
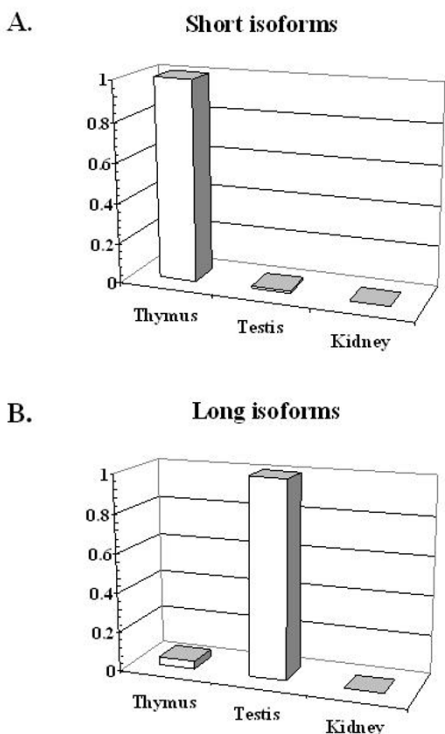
A.
Short isoforms

B.
Long isoforms

Figure 4
Expression of short and long *Spatial* mRNAs in mouse adult tissues by quantitative TaqMan RT-PCR. In each tissue, the expression levels of cDNA were normalised to the expression level of the housekeeping gene 18S-rRNA. **(A)** The ratios of short *Spatial* mRNAs/18S-rRNA from each tissue were standardised to short *Spatial* isoforms expression in the thymus which was taken as 1. Range of standard deviation: thymus (0.849–1.152); testis (0.009–0.014); kidney ($1.1\ 10^{-4}$–$1.4\ 10^{-4}$) **(B)** The ratios of long *Spatial* mRNAs/18S-rRNA from each tissue were standardised to long *Spatial* isoforms expression in the testis which was taken as 1. The tissues examined were: thymus, testis and the kidney chosen as negative control. Range of standard deviation: thymus (0.030–0.050); testis (0.839–1.191); kidney ($1.2\ 10^{-4}$–$1.4\ 10^{-4}$);

proline domain that could confer a specific function to this isoform.

Conclusions
We report the genomic characterization of the *Spatial* gene, which presents three short and two long splice variants. Here, a new *Spatial* isoform is characterized: it contains a sequence encoding for NLS protein signal and an

internal splice site producing a 69 bases exon 5 encoding a proline-rich domain. Short isoforms are highly expressed in the thymus by stromal cells which are involved in T cell development. Long isoforms are highly expressed by round spermatids in testis, and appear to be involved in a specific stage of spermatozoa differentiation. This unique gene, localized in B4 band of mouse chromosome 10, is well conserved in mammals and displays 5 different messengers generated by alternative splicing which is one of the main process for differential expression in mammals [21-23]. Future work is now required to understand *Spatial* specific tissues function and link alternatively spliced isoforms to specific promoter regulatory elements.

Methods
Animals
Mice C57BL/6 were maintained under specific-pathogen-free conditions and used between 4 to 8 weeks of age. All experimental and surgical procedures involving animals were approved by the veterinary office of the Ministry of agriculture, France.

RT-PCR
Total RNA was purified using TRIzol reagent (Gibco-BRL, Life Technologies). Single-strand cDNA was synthesized by reverse transcription on 0.3–5 µg of total RNA using oligo(dT)$_{25}$ and SuperScript II (Gibco-BRL) in a final volume of 20 µl. PCR reaction was performed in a PTC 200 Peltier Thermal Cycler (MJ research) using 2 µl of the RT reaction product in a final volume of 50 µl, using the following conditions: 94°C for 3 min (1 cycle), 30 cycles of 94°C for 30 s, 62°C for 40 s, 72°C for 1 min, and 72°C for 10 min (1 cycle). Of the amplification product, 10 µl were resolved on a 1% agarose gel. The sequence primers used to detect specifically Spatial-β are: forward primer: 5'-CTGAAGACAGGGAGGACA-3' and reverse primer 5'-CGCTGTCACCTTCGAGGTTA-3' while Spatial-α, -γ were amplified with forward primer: 5'-AGTCCAAAGGCA-GAGCCCCA-3' reverse primer 5'-CGCTGTCACCTTC-GAGGTTA-3' and the housekeeping gene GAPDH with forward primer: 5'-AACGACCCCTTCATTGAC-3' and reverse primer 5'-TCCACGACATACTC AGCAC-3'

Protein preparation and immunoblot analysis
To analyze the protein expression of Spatial-β, thymus from 4 weeks old mice was pounded. Nuclear and cytosolic proteins were separated by using the Nuclear Protein Extraction Kit (Panomics). Protein concentrations were measured using the Pierce BCA protein assay. For western-blot, total nuclear and cytosolic proteins were electrophoresed on SDS-polyacrylamide gel already prepared (Invitrogen) and transferred to Nitrocellulose membranes (BioRad) following incubation with the purified Spatial polyclonal antibody (1:2500). This antibody was

produced by immunizing two rabbits against the mouse recombinant 6 × His Spatial-α protein isoform and further purified on a protein G column (Eurogentec). Rabbit polyclonal anti-actin was used as a control charge (1:600; Santa cruz biotechnology). Proteins were visualized using horseradish peroxidase-conjugated secondary antibody (1:1000; Amersham Pharmacia Biotech) and the enhanced chemiluminescence (ECL) detection system (Pierce).

Quantitative TaqMan RT-PCR

Quantitative RT-PCR was performed on the thymus, testis and kidney chosen as a negative control to evaluate the distribution of the short and long *Spatial* isoforms by using the ABI PRISM 7000 Sequence Detection System. Random hexamers and the TaqMan reverse transcription reagents from the RT reaction mix (Applied Biosystems) were used to reverse transcribe total RNA. After, the PCR step was performed with TaqMan universal PCR master Mix and assays-on-demand gene expression probes (Applied Biosystems). Primers and the TaqMan probe used to detect specifically short isoforms were: forward primer: 5'-TTGGAACCAGCCCCTGTTT-3', reverse primer 5'-GTTCTCCGGCTTCGTCTCT-3' and FAM 5'-CCTTT-GGACTAGTCACCTCAT-3' NFQ, respectively. Primers and the TaqMan probe used to detect specifically long isoforms were: forward primer: 5'-GCTTCAAGAGCCTCAA-GAGACA-3', reverse primer 5'-GGTGGTGACCTAGTCTT CTTCAG-3' and FAM 5'-ACTGTAGGCTGCCTCTTG-3' NFQ, respectively. The 18S-rRNA was amplified from all samples on each plate as a housekeeping gene to normalize expression between different samples and to monitor assay reproducibility. A non-template control was included for each target analysed. Relative quantification of all targets was calculated by using the comparative cycle threshold method [24].

Fluorescence in situ hybridization

Metaphase spreads were prepared from a WMP female mouse, in which all the autosomes except chromosome 19 were in the form of metacentric Robertsonian translocations [25] Concanavalin A-stimulated lymphocytes were cultured at 37°C for 72 h with 5-bromodeoxyuridine added for the final 6 h of culture (60 μg/ml of medium) to ensure a chromosomal R-banding of good quality. The *Spatial* clone was biotinylated by random priming with biotin-14-dUTP, as outlined by the Gibco-BRL protocol (Life Technologies). Hybridization to chromosome spreads was performed with standard protocol. The biotin-labelled DNA was mixed with hybridization solution at a final concentration of 10 μg/ml and used 80 ng per slide. Before hybridization, the labelled probe was annealed with a 250-fold excess amount of murine Cot-1 DNA (Gibco-BRL) (for 45 minutes at 37°C) in order to compete the aspecific repetitive sequences. The hybrid-

ized probe was detected by means of fluorescence isothiocyanate-conjugated avidin (Vector laboratories). Chromosomes were counterstained and R-banded with propidium iodide diluted in antifade solution pH 11.0.

Sequence analysis

For the chromosomal localization, the genomic cosmid was isolated from rzpd genomic library http://www.rzpd.de. The human-mouse homology map was analysed with NCBI synteny database http://www.ncbi.nlm.nih.gov/Homology/. The *Spatial* corresponding contig was downloaded from the Ensembl Genome Browser http://www.ensembl.org. BLAST searches were conducted using the BLAST server http://www.ncbi.nlm.nih.gov/BLAST. The exact size and precise position of the start and the end of each exon for different *Spatial* isoforms are calculated using the AAT server http://genome.cs.mtu.edu/aat/aat.html. The sequence translation, exon organization, the multiple alignment and protein signature sequences have been analysed with Biology workbench http://workbench.sdsc.edu/.

Abbreviations

NCBI: National Centre of Biotechnology Information. RT-PCR: Reverse Transcription Polymerase Chain Reaction. BLAST: Basic Local Alignment Search Tool

Authors' contributions

MI contributed to the conception, design, and coordination; conducted the expression experiments, cloning, sequencing analysis, and. wrote parts of the manuscript.

DP participated in helpful discussion concerning the conception, and coordination of this study.

SG participated in helpful discussion concerning the revision and the sequence analysis.

FL participated to the analysis of mouse ortholog of *Spatial* gene.

GV participated to technical advises and maintenance of the laboratory.

MGM conducted the experiment of fluorescent *in situ* hybridization.

CN participated in the conception, designed, and coordination of the study and edited the manuscript.

All authors read and approved the final manuscript.

Additional material

Additional File 1

Figure 5: Word: document, sequence comparison of Spatial orthologs with the mouse protein. Sequence comparison of Spatial proteins in different species: Sus scrofa, Ss; Bos Taurus, Bt; Homo sapiens, Hs; Mus musculus, Mm and Rattus norvegicus, Rn. Residues conserved with the mouse protein are shown by (*), strongly conserved residues by (:) and weakly conserved residues by (.). Residues are colour coded: basic, DE, blue; acidic, KR, pink; polar, CGHNQSTY, green and hydrophobic, AFILMPVW, red. The start and the stop of translation for the different protein isoforms, are indicated by a red M and (*), respectively. A proline rich domain, specific of Spatial-β isoform, is highlighted in gray. Two positive amino acid rich domains are underlined. A putative nuclear localization signal is indicated, NLS and highlighted in yellow.
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2164-5-41-S1.doc]

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