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Molecular characterization of the murine homologue of the DC-derived protein DC-SCRIPT

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Abstract: Dendritic cell-specific transcript (DC-SCRIPT) is a putative DC zinc (Zn) finger-type transcription factor described recently in humans. Here, we illustrate that DC-SCRIPT is highly conserved in evolution and report the initial characterization of the murine ortholog of DC-SCRIPT, which is also preferentially expressed in DC as shown by real-time quantitative polymerase chain reaction, and its distribution resembles that of its human counterpart. Studies undertaken in human embryonic kidney 293 cells depict its nuclear localization and reveal that the Zn finger domain of the protein is mainly responsible for nuclear import. The human and the mouse genes are located in syntenic chromosomal regions and exhibit a similar genomic organization with numerous common transcription factor-binding sites in their promoter region, including sites for many factors implicated in haematopoiesis and DC biology, such as Gfi, GATA-1, Spi-B, and c-Rel. Taken together, these data show that DC-SCRIPT is well-conserved in evolution and that the mouse homologue is more than 80% homologous to the human protein. Therefore, mouse models can be used to elucidate the function of this novel DC marker. *J. Leukoc. Biol.* 79: 1083–1091; 2006.

Key Words: mouse · transcription factor · dendritic cells

INTRODUCTION

Dendritic cells (DC) are a group of motile bone marrow (BM)-derived cells that specialize in the uptake, processing, and presentation of antigens to T cells. At an immature state, DC act as sentinels in peripheral tissue continuously probing the antigenic environment. Upon antigen uptake, they migrate to lymph nodes where they present these antigens to T cells [1]. Through this interaction with T cells, DC tune the balance of our immune system towards immunity or tolerance, depending on the kind of antigens and the type of activating or inhibitory signals they have encountered in the periphery. DC can be categorized in different subtypes according to their phenotype, residual habits, or cellular origins. These DC subsets are best

defined in mouse, and mouse DC can be divided in plasmacytoid DC (PDC) and myeloid DC according to their precursor lineage. PDC are CD8⁺CD11b⁻ cells, and the myeloid lineage will generate CD8⁻CD11b⁺ cells (myeloid DC). Within the myeloid group, more subpopulations or activation stages can be discerned based on the expression of surface markers, such as CD4 and CD205 [2]. Langerhans cells are characterized by low levels of CD8, resembling lymphoid DC but also express CD11b, touching the side of the myeloid lineage. Similar to many developmental pathways, there are specific transcription factors that govern the divergence of different DC types. However, when it comes to DC development, only few such molecules have been described until now, such as PU.1 and Id2 [3, 4].

In an effort to unravel molecular pathways that render DC with this variability and antigen-presenting capacity, we searched for molecules specifically expressed in DC. Among others, we identified human DC-specific transcript (hDC-SCRIPT), a putative, transcriptional repressor containing a DNA-binding domain [11 C₂H₂ zinc (Zn) fingers], flanked by a proline-rich and an acidic region, which can interact with C-terminal-binding protein 1 (CtBP1), a global corepressor, suggesting a role for DC-SCRIPT in transcriptional repression [5]. Here, we describe the cloning of the murine counterpart of hDC-SCRIPT (mDC-SCRIPT), and we show that the protein is unique amid the family of transcription factors, as it resembles no other members outside the DNA-binding domain. The two proteins share more than 80% homology and bare strong similarities when it comes to sequence, genomic, organization, and expression patterns. In conclusion, DC-SCRIPT is a conserved molecule in DC, indicative for an important role in DC-immunobiology. The strong homology on protein and gene level between m- and hDC-SCRIPT will allow the application of murine models and knockout (KO) mice to study the precise function of this protein in DC.

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MATERIALS AND METHODS

Zoo blot analysis

The Zoo blot was hybridized in poly(ethylene glycol; PEG) buffer [10% PEG-8000, 7% sodium dodecyl sulfate (SDS)] at 50°C with a DC-SCRIPT-specific probe containing the most 3' of the open-reading frame (ORF) and part of the 3' untranslated region (UTR), obtained by polymerase chain reaction (PCR) and randomly labeled with ³²P-deoxy-cytidine 5'-triphosphate (T7 QuickPrime kit, Pharmacia, Uppsala, Sweden). The following primers were used for the PCR reaction: 5'-CTCAGGGCTTTTCAGAGTAC-3' and 5'-TCT-GGAAGAATACTCACAGTT-3'.

Cloning of mDC-SCRIPT

To clone mDC-SCRIPT, human primers were used on cDNA from BM-derived DC (BM-DC). The acidic region of mDC-SCRIPT was cloned by the following set of human primers: forward, 5'-CAGACAGCAAACCTTCAAG-3', and reverse, 5'-TACCGGATCCGATACCTAAAAGCACAGCTTG-3'. Similarly, for the murine proline-rich region, the following human primers were used: forward, 5'-GGGTCTAGGAAACCGAAGAGC-3', and reverse, 5'-TCTCCACCTTCTGCTTGGTC-3'. Once the acidic and the proline-rich regions were cloned and sequenced, specific primers were designed for the cloning of the Zn finger region (forward: 5'-TCAGCAGGCACACCTTCCTG-3', reverse: 5'-ACCTTGAGAGTGCAGGCCTCG-3'). The remaining 5' part of mDC-SCRIPT was cloned with standard rapid amplification of cDNA ends PCR techniques (Roche, GmBH, Mannheim, Germany).

Cells, animals, and culture conditions

Human embryonic kidney (HEK) 293 T cells were cultured in Dulbecco's modified Eagle's medium (Gibco-BRL Life Technologies, Grand Island, NY), supplemented with 10% heat-inactivated fetal calf serum (FCS; Gibco-BRL Life Technologies), 10 nM 2-N-hydroxyethylpiperazine-N'-2-ethanolsulfonic acid, pH 7.7 (HEPES, Boehringer Mannheim GmbH, Germany), 0.1 mM minimal essential medium nonessential amino acids, and 100 units/ml anti-biotic-antimycotic (both from Gibco-BRL Life Technologies).

BM-DC were prepared as described previously [6]. Briefly, BM cells were collected at Day 0 from C57BL/6 mice (Charles River Laboratories, Wilmington, MA), and 10⁶ cells/well were cultured in six-well plates using RPMI 1640 (Life Technologies, Inc., Gaithersburg, MD), 5% FCS with murine recombinant granulocyte macrophage-colony stimulating factor (mGM-CSF; 20 ng/ml, PeproTech Inc., Rocky Hill, NJ), and murine recombinant interleukin (mrIL)-4 (20 ng/ml, DNAX, Palo Alto, CA). At Days 3 and 6, fresh medium containing the adequate cytokines was added. Maturation was induced at Day 7, using lipopolysaccharide (LPS; 2 µg/ml). At Day 8, nonadherent cells were collected.

For obtaining splenic DC, spleens were collected, chopped, and digested at 37°C with collagenase type 3 (1 mg/ml, Worthington Biochemical Corp., Freehold, NJ) and DNase I (20 µg/ml, Boehringer-Mannheim) for 20 min. EDTA (10 mM) was added, and the cellular suspension was separated into low- and high-density fractions on a Nycodenz gradient (Nycodenz Pharma, UK). The recovered low-density fraction was cultured overnight or purified by incubation with anti-CD11c-coupled microbeads and a positive selection over a magnetic cell sorter (MACS[®]) column (Miltenyi Biotec, Auburn, CA) to obtain immature DC (iDC). The negative fraction was also collected and named CD11c⁻. After overnight culture, nonadherent cells contained at least 90% of DC, as assessed by morphology and specific staining, using the anti-CD11c monoclonal antibody (mAb) N418. These cells were considered as mature DC (mDC) [7].

Reverse transcriptase (RT)-PCR and real-time PCR

Total RNA was extracted from 5–10 × 10⁶ cells using Trizol reagent (Life Technologies, Inc.) and subsequently transcribed into cDNA using an oligo-dT primer, random hexamers, and the Moloney murine leukemia virus RT (Life Technologies, Inc.). As a control, the reactions were also performed in the absence of RT. Real-time PCR reactions were performed in duplicate using an end concentration of 125 nM probe and 300 nM primers. The amplifications were performed on an ABI/PRISM 7700b sequence detector system (PE-Applied Biosystems, Foster City, CA). A DC-SCRIPT-specific probe (TGAACCTACGCCCCACAGATCCCG) was labeled at the 5' end with carboxy-

fluorescein and at the 3' end with carboxytetramethylrhodamine (TAMRA). The specific probe for the rodent housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (rodent GAPDH; TaqMan[®] rodent GAPDH control reagents, PE-Applied Biosystems) was labeled at the 5' end with a VIC fluorescent group and at the 3' end, with TAMRA. The TaqMan[®] rodent GAPDH control reagents were used for the detection of rodent GAPDH, and DC-SCRIPT was amplified using the forward primer 5'-CACCTCAAGCAACTCACT-3' and the reverse primer 5'-CACTGGTAGGCACGGATGTTG-3'. Calculations were performed as described previously [8]. The amount of DC-SCRIPT expressed was normalized to GAPDH.

Plasmids, transfections, immunoblotting, and immunocytochemistry

FLAG constructs were cloned in the pCATCH vector [9]. HEK 293 T cells (8 × 10⁵) were plated in six-well plates and transfected with 10 µl LipofectAMINE 2000 (Gibco-BRL Life Technologies) and 1 µg DNA. Cells were harvested 1 day after transfection. Whole cell lysates were prepared in 1% SDS standard lysis buffer. Equal amounts of protein were separated by SDS-polyacrylamide gel electrophoresis, and proteins were transferred to Protran nitrocellulose transfer membrane (Schleicher and Schuell BioScience, Keene, NH). A combination of M2 mouse anti-FLAG (1 µg/ml, Sigma Chemical Co., St. Louis, MO) and horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G (IgG; H⁺L) antibody (0.4 µg/ml, Pierce, Rockford, IL) was used to detect FLAG-tagged proteins. For immunofluorescent staining, HEK 293 T cells were seeded on eight-chamber slides (Nunc, Rochester, NY), coated with fibronectin (20 µg/ml, Roche). Cells were fixed in cold methanol and blocked with 3% bovine serum albumin (Calbiochem, San Diego, CA) in phosphate-buffered saline. M2 mouse anti-FLAG (Sigma Chemical Co.) and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG, (H⁺L, Molecular Probes, Junction City, OR) were used for detection. Nuclei were stained with propidium iodide (PI). Slides were mounted with Vectashield (Vector Laboratories, Burlingame, CA) and analyzed by confocal laser scanning microscopy (CLSM; Biorad 100).

CONREAL database searches

For comparing the promoter regions of m- and hDC-SCRIPT, both promoters were retrieved from the ENSEMBL database (within the CONREAL site), and 1500 base pairs (bp) upstream of the translation initiation site were compared. The thresholds used were a higher homology than 75% for binding of the corresponding transcription factor between mouse and human, and the relative score was higher than 0.8 while using 15 flanking bp for calculating homology.

RESULTS

DC-SCRIPT is conserved in evolution

Recently, we have described the cloning of hDC-SCRIPT, a novel DC marker in humans [5]. To determine whether the single-copy gene encoding hDC-SCRIPT is conserved in evolution, genomic DNA from human, chicken, mouse, hamster, and pig was digested with *EcoRI*, separated on an agarose gel, and hybridized with a hDC-SCRIPT-specific cDNA probe covering the 3' end of the ORF. Specific bands could be detected in all species tested, including human (**Fig. 1**). Further searches in silico revealed predicted transcripts of DC-SCRIPT in many other species, apart from human and mouse (rat, chimpanzee, chicken, pufferfish). A homologous protein to DC-SCRIPT was also described for *Fugu rubripes* and named fzfl [10]. Comparison of these transcripts shows that the DC-SCRIPT protein is well-conserved between species, especially the putative DNA-binding domain (11 C₂H₂ Zn fingers) in the different orthologs, which is almost identical (>85% identity), suggesting that the protein could bind the same DNA sequence in these different species.

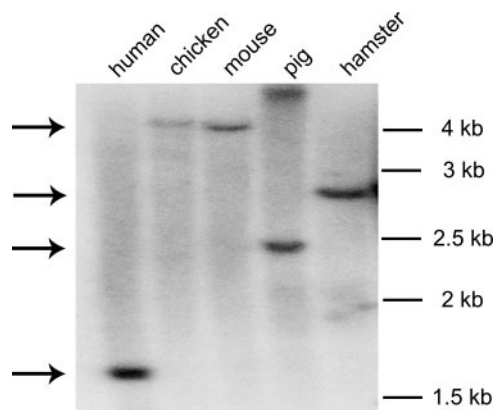


Fig. 1. Zoo blot analysis of DC-SCRIPT using genomic DNA derived from human, chicken, mouse, pig, and hamster origin.

DC-SCRIPT protein shares high homology between mouse and human

We cloned the cDNA-encoding mDC-SCRIPT [746 amino acids (aa)] from mDC. mDC-SCRIPT encodes a protein similar to hDC-SCRIPT and contains a proline-rich region (aa 106–216), 11 C₂H₂ Zn fingers (aa 242–553), and an acidic region close to the C-terminus (aa 583–700; **Fig. 2**). Like hDC-SCRIPT, there is a consensus NLS in positions 77–80. Multiple consensus phosphorylation sites are present throughout mDC-SCRIPT. Although transcription factors have never been shown to be myristoylated, mDC-SCRIPT bears three myristoylation motifs, all of which are gathered in the acidic region

of the protein (564–569 GLGRGR, 577–582 GVLRNL, 711–716 GQGPSF). In human, only the first two are conserved. Furthermore, there are three sites for N-glycosylation (108–111 NLTL, 391–394 NCSE, 544–547 NLTR). Up to date, there is only one transcription factor that has been shown to be N-glycosylated [11] still with unclear meaning. Two out of three N-glycosylation sites are situated within the Zn fingers, and both sites are conserved between mouse and human. In addition, the two CtBP1-binding motifs, which are found in human, are also present in mDC-SCRIPT (aa 587–591 and 646–650). Both of them are identical to human, and in human only the first one is responsible for the interaction.

Alignment of the two proteins (**Fig. 3**) shows that the proline-rich region and the Zn fingers are highly homologous to that of the human ortholog (92.2% identity). It is remarkable that the Zn fingers are identical in both proteins, implying that both orthologs bind the same DNA sequence and possibly regulate the same genes in both species. The acidic region shares the least homology between the two species (56.8% identity). The murine acidic region contains four amino acids in surplus compared with hDC-SCRIPT. However, these residues do not seem to fall into any particular motif or affect the biochemical characteristics of this region.

Genomic organization of m- and hDC-SCRIPT

The homology of the two orthologs extends beyond the protein level to gene and chromosomal organization. The murine gene of DC-SCRIPT is spanning a region of 62 kb on chromosome 13 (13 D1) between 95,924 kb and 95,987 kb (**Fig. 4A**). The gene consists of five exons, where the first exon encodes for a

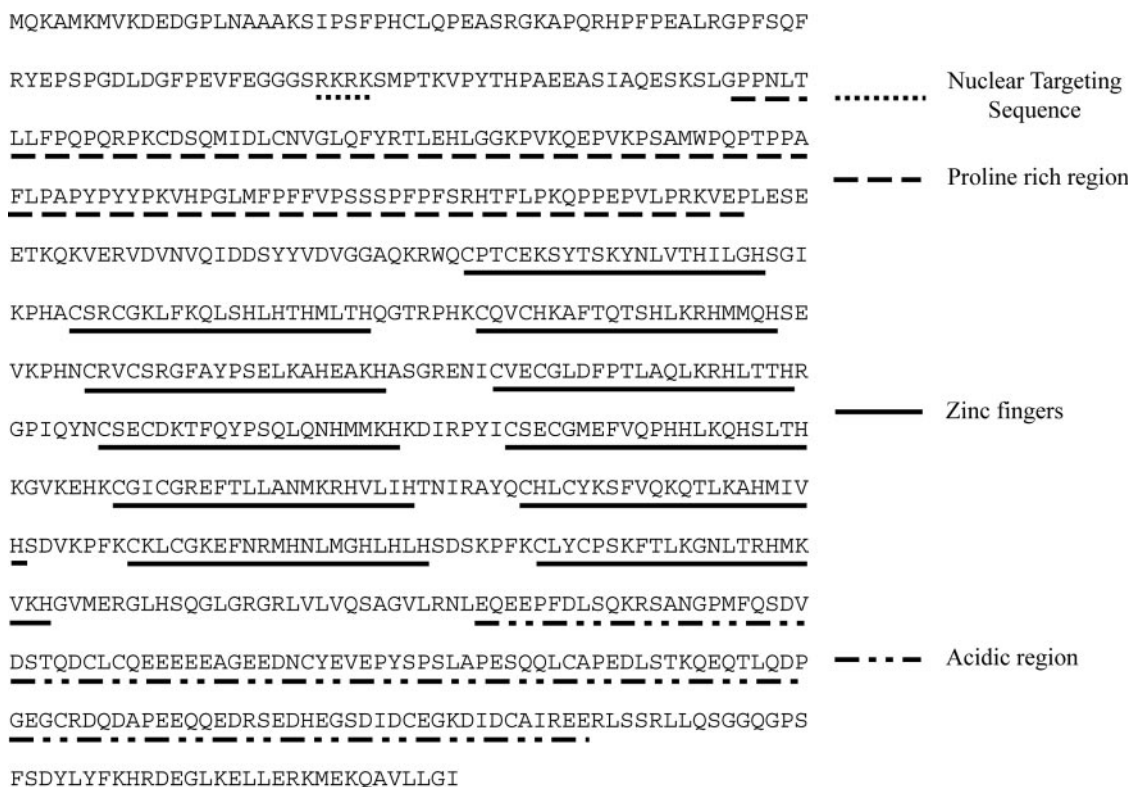


Fig. 2. Protein sequence and different regions of mDC-SCRIPT. The nuclear localization sequence (NLS) is noted as are the proline-rich region, the 11 C₂H₂ Zn fingers, and the C-terminal region full of negatively charged residues.

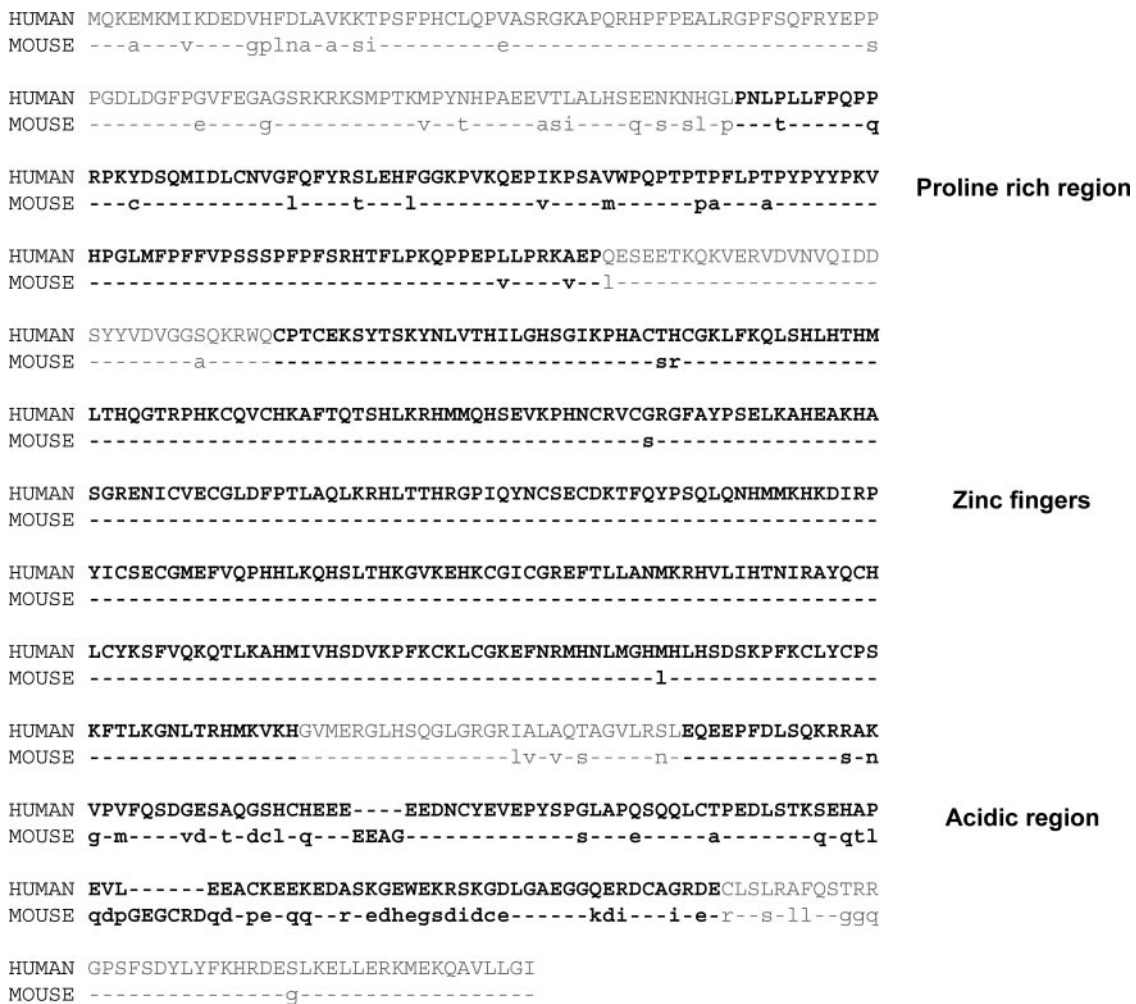


Fig. 3. Sequence alignment of the m- and hDC-SCRIPT orthologs. The different regions of the molecule are also indicated. In the mouse, hyphens (-) represent identical amino acids, and nonidentical amino acids are indicated in lowercase letters. In the human sequence, hyphens indicate stretches of exceeding amino acids in the mouse sequence. These amino acids are in capital letters in the mouse ortholog. Proline-rich region, Zn fingers, and acidic region are shown in bold letters.

5' UTR, and the remaining exons give rise to the ORF. Half of the ORF is encompassed in the second exon, and the next two encode for two Zn fingers each. The last exon covers the acidic region and an extended 3' UTR. In Figure 3, it is evident that the acidic region displays the most diversity between the two species. It is interesting that this region is encoded by a sole exon. The boundaries between introns and exons fall into the 3' splice site (ss) GT-5' ss AG rule. hDC-SCRIPT has exactly the same chromosomal organization on the large branch of chromosome 5 (5q 13.2, Fig. 4A). The chromosomal regions 13D in mouse and 5q13 in human are syntenic, and the neighboring genes of DC-SCRIPT are also well-conserved. These genes include transportin 1, pentatricopeptide repeat domain 2, mitochondrial ribosomal protein S27, and microtubule-associated protein 1B. One difference between the murine and the human gene is that transcription of the human gene runs from the telomere toward the centromere, and that is reversed in mice.

Next, we compared 1500 bp upstream of the first exon of the h- and mDC-SCRIPT gene by use of the CONREAL database [12]. Comparison of the putative promoter region also revealed striking similarities in the presence of consensus sequences for transcription factor binding (Fig. 4B). These binding sites have

a diverse probability for recruiting transcription factors. Most of these conserved sites are clustered in the 300 bp preceding the first exon. It is interesting that transcription factors with a profound role in hematopoiesis and DC biology can bind with high probability in this region in mouse and human, such as growth factor independence (Gfi), GATA-1, activated protein-1 (AP-1), Spi-B, nuclear factor (NF)-κB, and c-Rel (**Table 1**).

Expression pattern of mDC-SCRIPT in DC

To investigate expression of mDC-SCRIPT in leukocytes, semi-quantitative, real-time PCR analysis was carried out on BM and spleen cells (**Fig. 5**). BM cells were cultured with GM-CSF plus IL-4 for 8 days to generate DC. At Days 0 (no culture), 3, 7, and 8, nonadherent cells were collected. To induce DC maturation, LPS (2 μg/ml) was added at Day 7 for the last 24 h. mDC-SCRIPT mRNA levels are already detectable at 3 days of culture in the presence of GM-CSF and IL-4 and remain elevated for the remaining culture period (Fig. 5A). The data further show that maturation induced by LPS has no or little effect (two-fold) on DC-SCRIPT mRNA levels relative to IDC (Fig. 5).

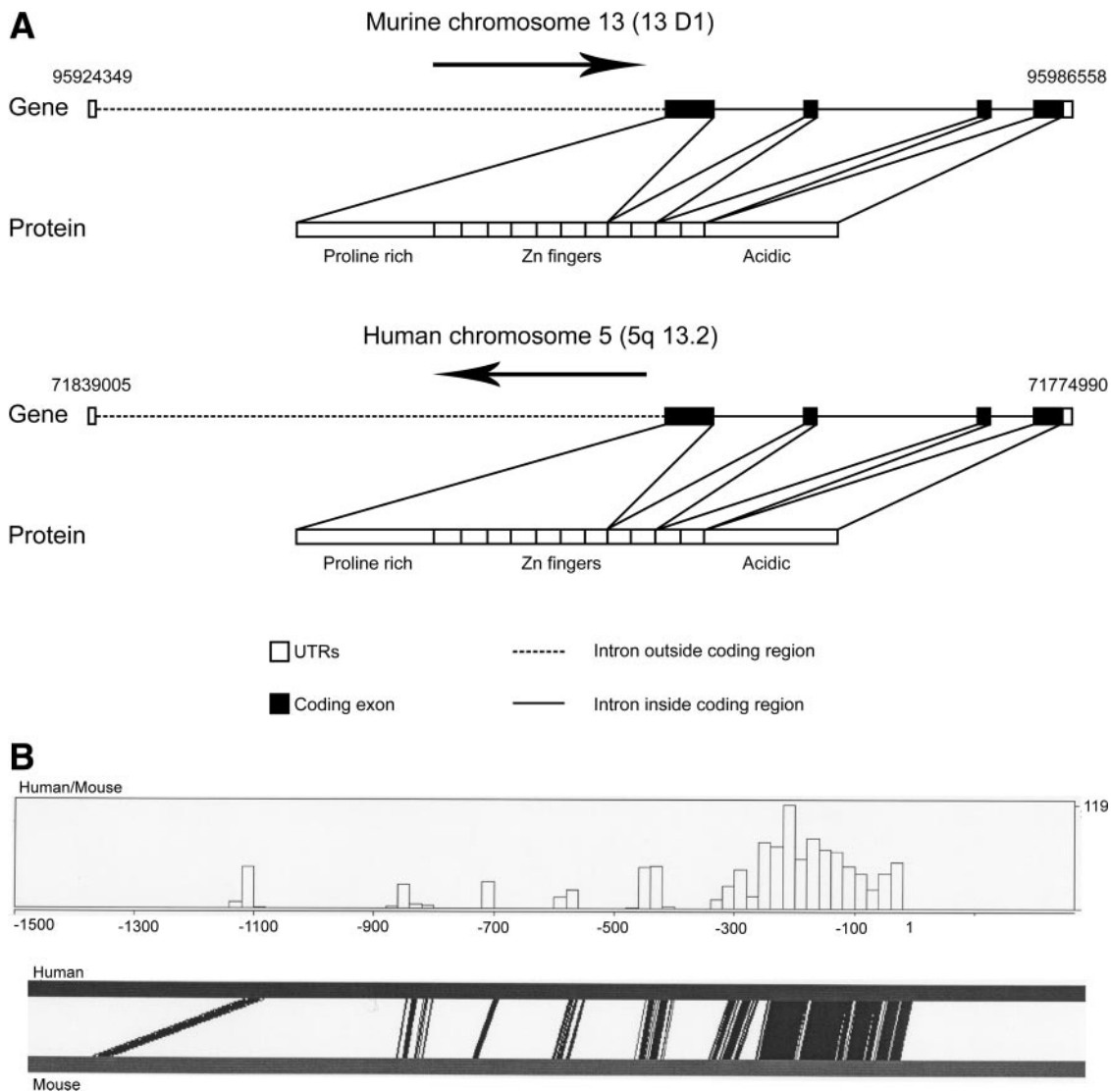


Fig. 4. (A) Chromosomal organization of the m- and hDC-SCRIPT genes. Introns are represented as lines and exons as boxes. UTRs and coding sequences are marked. The arrows show the direction of the chromosome from centromere to telomere. The boundaries of the gene on the chromosome are indicated on top of the gene, whereas the protein is depicted underneath. Regions of the protein that are encoded from each exon are shown as well. (B) Analysis of the 1500-bp region upstream of the first exon for both orthologs. The graph in the upper panel shows the number of putative common transcription factors that bind to mouse and human sequences. Each bar represents the number of common factors that bind to the specific site (site number is shown under the graph). The lower panel is a graphical representation of the upper. The mouse and human sequence are aligned relevant to common transcription factor-binding sites. The sequences are depicted as horizontal bars, and the black lines represent transcription factor-binding sites between the two sequences.

To determine whether DC-SCRIPT was expressed specifically in freshly isolated mDC, we performed real-time semi-quantitative PCR on cDNA from total spleen cells (Fig. 5B, SC; containing 1–3% of DC) or different splenic, DC-enriched fractions: recovered low-density fraction (LD) containing ~20% DC, CD11c⁺ MACS-purified, IDC (>98%), or purified MDC. CD11c⁻ cells were used as a negative control (N418⁻). DC-SCRIPT expression was readily detected in IDC and remained unchanged after spontaneous maturation (MDC), whereas mRNA coding for DC-SCRIPT was detected in the low-density fraction and total spleen cells at lesser amounts. The seemingly equal levels of DC-SCRIPT between the spleen cell and low-density cell populations can be explained with variable levels of DC in the spleen cell populations as well as variable expression of GAPDH in other cell populations in

spleen, which could affect the normalization. Conversely, the CD11c⁻ (N418⁻) cells do not express detectable levels of DC-SCRIPT (Fig. 5B, middle bar).

Localization of mDC-SCRIPT

Analysis of the mDC-SCRIPT protein was performed by a series of FLAG-tagged constructs, each encoding part of the DC-SCRIPT protein. HEK 293 T cells were transiently transfected and analyzed by Western blotting and CLSM using the M2 anti-FLAG mAb (Sigma Chemical Co.). As shown in **Figure 6A**, all constructs were correctly expressed and encoded proteins of the expected size. For CLSM purposes, cells were cultured on fibronectin-coated slides and stained with the M2 anti-FLAG antibody. Similarly to hDC-SCRIPT, full-length mDC-SCRIPT was localized to the nucleus of the cells, as

TABLE 1. Putative Common Transcription Factor-Binding Sites in the Promoters of m- and hDC-SCRIPT

Transcription factor	Binding site	Binding-site human	Binding-site mouse	Identity %	Rel. score human	Rel. score mouse
GATA-1	SNNGATNNNN	-120	-139	95.35	0.81	0.81
		-204	-223	97.50	0.81	0.81
		-227	-246	100	0.93	0.93
AP-1	RSTGACTNANW	-81	-100	92.68	0.88	0.88
		-130	-149	100	0.81	0.81
Spi-B	WSMGAA	-19	-38	89.19	0.83	0.83
		-25	-44	97.30	0.86	0.86
		-99	-118	91.89	0.97	0.97
		-123	-142	94.59	0.83	0.83
		-209	-228	100	0.89	0.89
		-441	-458	91.89	0.86	0.92
NF- κ B	GGGAMTTYCC	-289	-320	82.50	0.82	0.83
c-Rel	SGGRNTTCC	-289	-320	82.50	0.85	0.85
Ikaros 2	NTGGGAWNNC	-139	-158	100	0.91	0.91
		-192	-211	97.62	0.85	0.85
Gfi	YMAATCWSWS	-71	-90	90	0.82	0.81

revealed by simultaneous PI staining of DNA (Fig. 6B). The absence of the proline-rich region that bears the putative NLS did not affect the nuclear localization of the protein. However, nuclear localization was abolished upon deletion of the Zn fingers of mDC-SCRIPT, and the acidic region alone is preferentially located in the cytoplasm.

Next, the role of the consensus NLS in the proline-rich region and the Zn fingers in the nuclear localization of DC-SCRIPT were analyzed. In Figure 6B, it is evident that the proline-rich region alone localized mainly to the cytoplasm and in some cells, also partly to the nucleus. Similar results were obtained with the human proline-rich region (Fig. 6B, seventh panel). In the presence of the NLS of simian virus 40, however, the proline-rich region efficiently localizes to the nucleus, implying that the consensus NLS in mDC-SCRIPT is a weak nuclear localization signal at best. In contrast, the Zn finger region alone is sufficient for nuclear localization. We note that

the diffuse nuclear staining observed with the proline-rich region does not colocalize with PI, whereas the staining observed for the Zn fingers does colocalize with PI and has a more dotted appearance. These data imply that the Zn finger region but not the proline-rich region of DC-SCRIPT associates with nuclear niches of high DNA abundance, which are PI⁺.

DISCUSSION

DC are the most potent antigen-presenting cells of the immune system, and different subpopulations of DC are derived from lymphoid or myeloid progenitors. Still, little is known about the transcription factors that are involved in the development and activation of DC. Recently, we identified a novel transcription factor preferentially expressed in hDC, DC-SCRIPT. Herein, we report the cloning and characterization of the murine or-

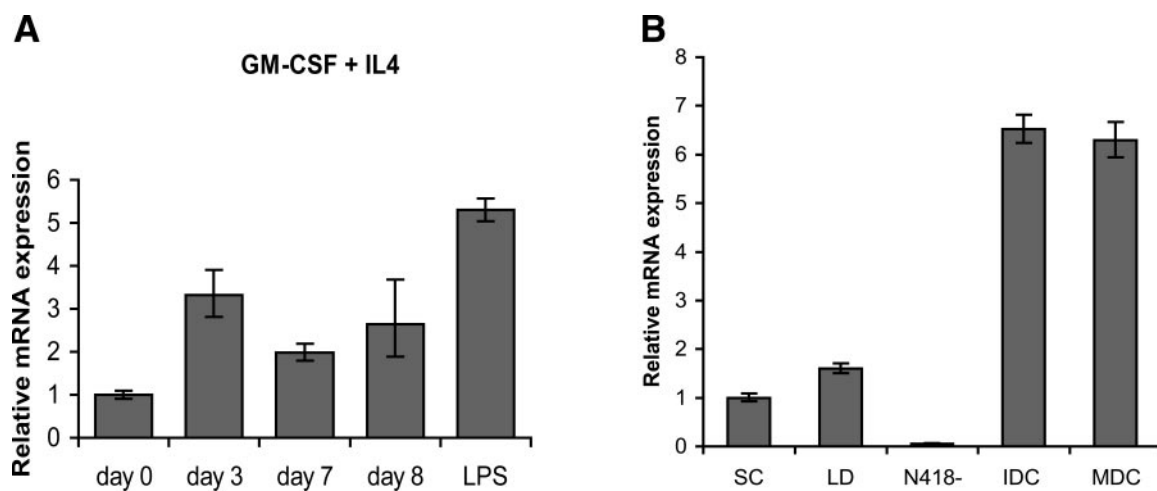


Fig. 5. Relative mRNA expression of DC-SCRIPT by distinct DC subsets. (A) The levels of DC-SCRIPT mRNA expression were examined in fresh BM cells (Day 0), immature BM-DC (Days 3, 7, and 8), or LPS-matured BM-DC (LPS). The BM cells were cultured with mrGM-CSF and mrIL-4. Total RNA (5 μ g) was transcribed into cDNA, and real-time PCR was performed as indicated in Materials and Methods. (B) cDNA from spleen cells (SC), low-density cells (LD), CD11c⁻-recovered low-density cells (N418⁻), IDC, and MDC was used to measure the expression of DC-SCRIPT as described in Materials and Methods. The data shown are the mean \pm SD of duplicates. Three independent experiments were performed with similar results.

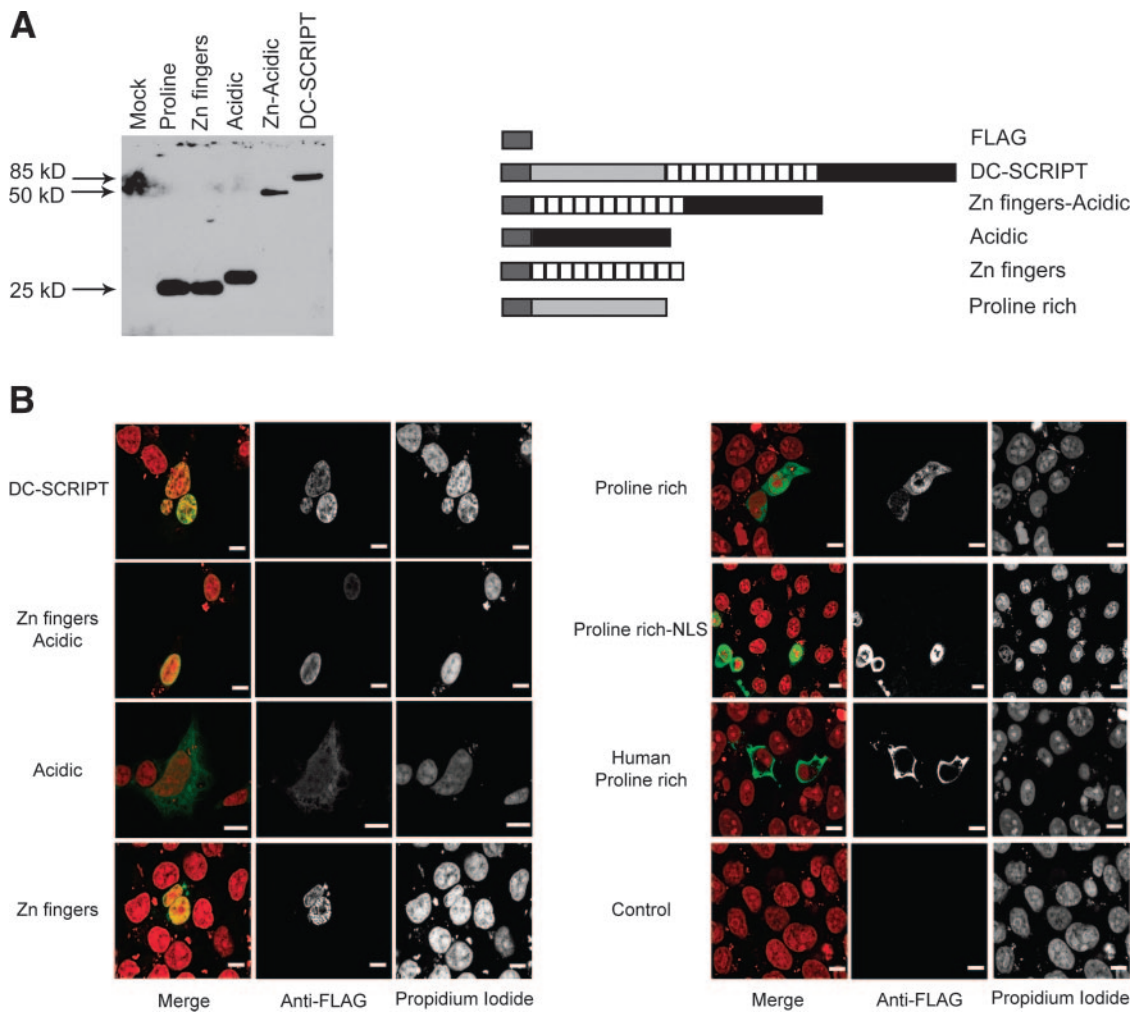


Fig. 6. Protein expression and localization of mDC-SCRIPT. (A) Protein expression of different constructs of DC-SCRIPT in HEK 293 T cells. The approximate size of each band is indicated by an arrow. Constructs are schematically shown on the right as well. (B) Localization pattern of these constructs in HEK 293 T cells. Nuclei were stained with PI (right column), and mDC-SCRIPT constructs were visualized by means of FITC-coupled anti-FLAG mAb (middle column). The merged picture obtained from the CLSM and the corresponding construct are shown on the left. Original bars, 5 μ m.

tholog of hDC-SCRIPT and show that the two genes and proteins are highly homologous.

mDC-SCRIPT is also an 11 C₂H₂ Zn finger protein with a proline-rich and an acidic region flanking the Zn fingers. DC-SCRIPT is well-conserved in evolution (Fig. 1), and the homology between h- and mDC-SCRIPT exceeds 80%. The region of highest homology is the Zn fingers, which are 98.7% identical, and preliminary data indeed demonstrate that m- and hDC-SCRIPT recognize the same DNA stretch. Outside the Zn fingers, homology levels are somewhat lower but still strikingly high and include the CtBP-binding sites [5] (aa 587–591, 646–650) and consensus protein modification motifs, such as potential phosphorylation sites. Recruitment of CtBP1 by DC-SCRIPT could lead to silencing of its target genes. It seems therefore that m- and hDC-SCRIPT use the same pathway to control expression of their target genes. mDC-SCRIPT preserves both putative binding sites for CtBP1 in exactly the same order and region as hDC-SCRIPT. In the case of the human ortholog, it has been shown that only the first site is responsible for attachment of CtBP1 to DC-SCRIPT [5]. In addition, phosphorylation motifs are of vital significance to

transcription factors, as they have been shown to modify their function, localization, and stability [13, 14].

The m- and hDC-SCRIPT genes also share the same intron-exon organization and chromosomal arrangement, including neighboring genes. It is intriguing that comparison of the 5' upstream region of each gene revealed a cluster of transcription factor-binding sites, which are similar between mouse and human. These sites occupy a stretch of approximately 300 bp upstream of the predicted transcription initiation site. Although the comparison was made in silico, some of the transcription factors that can bind to this locus are of central importance to DC biology. For example, Gfi was shown recently to favor DC development from precursors and also to play an important role in maturation [15]. c-Rel regulates IL-12 p70 expression in CD8⁺ DC [16], and NF- κ B controls various pathways in DC [17, 18]. It is interesting that c-Rel and NF- κ B bind the same region. It could be that under certain conditions or signals, one of the two factors is binding or only one of the two is really active on that site. Spi-B has been shown to orchestrate PDC differentiation [19]. What is more relevant to DC biology is the combination of these transcription-factor

binding sites. The balance between NF- κ B and Jun N-terminal kinase/AP-1 is crucial for DC viability [18], and the interaction amid GATA-1 and PU.1 directs the final stages of haematopoiesis. GATA-1 inhibits PU.1 by preventing it from interacting with its essential coactivator c-Jun [20]. The presence of consensus-binding sites for the aforementioned transcription factors in the promoter of mDC-SCRIPT is consistent with a prominent role of mDC-SCRIPT in DC. Indeed, semiquantitative real-time PCR analysis demonstrated that mDC-SCRIPT is preferentially expressed among leukocytes in freshly isolated DC from spleen as well as in BM-DC.

mDC-SCRIPT as well as the human ortholog contain 11 C₂H₂ Zn fingers, and Zn finger proteins belong mainly to the family of transcription factors, with the Zn fingers being the DNA-binding region of the protein. In many cases though, Zn fingers can mediate protein–protein interactions or even protein–RNA interactions [21]. This holds true, especially for proteins with multiple Zn fingers such as CCCTC-binding factor [22]. In such proteins, Zn fingers are involved mainly in post-transcriptional processes such as maturation, nuclear export, and stabilization of mRNA. We have recently obtained data suggesting that DC-SCRIPT, the human as well as the murine ortholog, can bind directly to a specific DNA sequence (unpublished data). However, we cannot exclude the possibility that DC-SCRIPT engages some Zn fingers in DNA binding, and the others are coupled to different functions (e.g., nuclear translocation). Initial characterization of the mDC-SCRIPT protein indicates that it localizes to the nucleus. Truncation analysis of the protein revealed that similar to hDC-SCRIPT, the Zn fingers are sufficient for nuclear localization. It is striking that the consensus NLS, present in the proline-rich region, does not appear to be of major importance for nuclear localization, as the proline-rich region alone shows cytoplasmic as well as nuclear staining (Fig. 6B). Possibly, mDC-SCRIPT uses two different mechanisms for nuclear import, acting cooperatively to drive and maintain the protein in the nucleus. One mechanism involves the native NLS and importins [23], and the second involves the Zn fingers. As the latter region lacks an apparent NLS sequence, the Zn fingers may bind to another protein that drives the nuclear transport of DC-SCRIPT, analogous to pancreatic transcription factor 1 [24]. Furthermore, the Zn fingers may enhance nuclear retention. The staining patterns of DC-SCRIPT and the Zn fingers reveal patches within the nucleus that correlate with PI staining. Such a pattern can be attributed to cooperating molecules, which preferentially drag the molecule to certain niches, or it could reveal a preference of the molecule for anchoring to certain promoters and controlling expression of its target genes. As shown for the androgen receptor, abrogation of its DNA-binding capacity causes the molecule to diffuse in the nucleoplasm, the wild-type protein displays a dotted localization pattern [25].

Collectively, our data indicate that the murine ortholog of DC-SCRIPT is not only similar to human, but it is controlled likewise and possibly has the same function in DC. Especially in haematopoiesis, many transcription factors are conserved from teleosts to mammals. Similar combinations of transcription factors govern a like developmental stages [26]. DC-SCRIPT could be one of the factors that directs DC develop-

ment. The high degree of conservation between m- and hDC-SCRIPT implies that mouse models, including KO mice, may be used to unravel the function of DC-SCRIPT.

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