Chromosomal characterization and localization of the NAD$^+$-dependent histone deacetylase gene *sirtuin 1* in the mouse

ULRICH MAHLKNECHT and SUSANNE VOELTER-MAHLKNECHT

Saarland University Medical Center, Department of Internal Medicine, Division of Immunotherapy and Gene Therapy, José Carreras Research Center, Kirrberger Str., D-66421 Homburg/Saar, Germany

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Abstract. Sirtuin 1 (SIRT1) is a nicotinamide adenine dinucleotide (NAD$^+$)-dependent deacetylase, which belongs to the silent information regulator 2 (Sir2) family of histone deacetylases (HDACs). The yeast Sir2 protein and its mammalian derivatives play a central role in epigenetic gene silencing, DNA repair and recombination, the cell cycle, microtubule organization, and in the regulation of aging. We isolated and characterized the murine *Sirt1* genomic sequence, which spans 19,910 bp and has one single genomic locus. Determination of the exon-intron splice junctions established that SIRT1 is encoded by 9 exons ranging in size from 80 bp (exon 6) to 1,927 bp (exon 9). Characterization of the 5' flanking genomic region, which precedes the *Sirt1* open reading frame, revealed a number of NFκB and GATA transcription factor binding sites in addition to a 393-bp CpG island. The 3,882-bp murine *Sirt1* transcript has an open reading frame of 2,211 bp and encodes a 737 aa protein with a predicted molecular weight of 80.4 kDa and an isoelectric point of 4.60. Next to this transcript, a shorter, 3,765 bp splice variant with an open reading frame of 2,094 bp, that lacks exon 2 and encodes a 698 aa protein with a predicted molecular weight of 76.0 kDa and an isoelectric point of 4.62 has been reported. Fluorescence *in situ* hybridization analysis identified a single genomic locus for the murine *Sirt1* gene on chromosome 10 B4 and is neighbored by the *Herc4* and *Dnajc12* genes.

Correspondence to: Dr Ulrich Mahlknecht, Saarland University Medical Center, Department of Internal Medicine, Division of Immunotherapy and Gene Therapy, José Carreras Research Center, Kirrberger Str., Bldg. 45.3, D-66421 Homburg/Saar, Germany
E-mail: ulrich.mahlknecht@uks.eu

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Introduction

Based on phylogenetic analyses, structural and functional similarities, mammalian histone deacetylases (HDACs) are grouped into four subclasses, which comprise three classes of non-sirtuin HDACs, i.e. the yeast histone deacetylase RPD3 homologs (class I HDACs), the yeast histone deacetylase HDA1 homologs (class II HDACs), HDAC11 as the only class IV HDAC, and the sirtuins (class III HDACs), which received their name based on their homology with the yeast Sir2 protein. So far, seven human and murine sirtuins have been identified (SIRT1-7) (1-3).

The main characteristic feature that distinguishes the sirtuins from other HDACs, is their unique enzymatic mechanism. While the class I, II and IV HDACs are Zn^{2+}-dependent hydrolases, the sirtuins possess a unique nicotinamide adenine dinucleotide (NAD$^+$)-dependent protein deacetylase and in some cases a secondary ADP-ribosyltransferase activity (4,5). Derivatives of the yeast Sir2 HDAC share a common catalytic domain, which is highly conserved throughout evolution and which is composed of two distinct motifs that bind NAD$^+$ and the acetyl-lysine substrate, respectively (6). This deacetylase domain is being flanked by N- and C-terminal extensions of variable length, which are characterized by a considerable variance suggesting the acquirement of new functions through evolution. Also, there is increasing evidence that the N- and C-terminal ends of mammalian sirtuins are required for the regulation of subcellular localization and/or catalytic activity (7-10).

Sirtuin 1 (SIRT1) is known to deacetylate histone H3 Lys_9 and Lys_{14} and specifically histone H4 Lys_{16}, while it hydrolyzes one molecule of NAD$^+$ for every lysine residue that is deacetylated (11). SIRT1, like Sir2, is known to directly modify a number of different cellular processes such as heterochromatin silencing (9,12-14), the modulation of meiotic checkpoints (15), and as a potential antiaging effect, increasing genomic stability and suppressing rDNA recombination (12,16). Also, it modifies differentiation, metabolism, neuronal protection, apoptosis and cell survival due to its ability to deacetylate both histone and numerous non-histone targets (17). Additionally to its critical role in tumorigenesis

due to its participation in cellular stress response, there is emerging evidence for SIRT1 being a suitable cancer target. siRNA-mediated knockdown of SIRT1 has for instance been shown to induce growth arrest and apoptosis in human epithelial cancer cells whereas normal epithelial cells remained unaffected (18,19).

In yeast, Sir2 has shown to connect cellular energy levels with longevity (12). Calorie restriction induces a metabolic switch that either increases the NAD/NADH ratio and/or decreases nicotinamide, which is a Sir2 inhibitor and therefore enhances Sir2 and increases rDNA stability (12,20). Also, during calorie restriction in mammals SIRT1 has been reported to activate fat mobilization in white adipocytes and repress genes that mediate fat storage (21). The over-expression and/or activation of yeast Sir2 homologs, and SIRT1 in particular, has been reported to mediate a deacetylation-dependent inhibition of downstream activities, which not only include the tumor suppressor p53 and the FOXO transcription factor family, but also the p65/RelA subunit of nuclear factor-κB (NF-κB) (22) and to extend the life span in various organisms (23-25). The SIRT1 protein deacetylates p53 and thus reduces its activity (26,27), an effect, which is inhibited by nicotinamide. In addition, SIRT1 reduces the activity of the mammalian forkhead transcription factor FOXO3A and thus reduces both forkhead-dependent and p53-dependent apoptosis in response to DNA damage and oxidative stress (28). On the other hand, acute nutrient withdrawal in mammalian cells activates FOXO3A and enhances FOXO3A-mediated expression of SIRT1. SIRT1 is known to exhibit a dual effect on FOXO3 function: while SIRT1 increases the FOXO3 ability to induce cell cycle arrest and resistance to oxidative stress, it also inhibits FOXO3-induced apoptosis, which consequently results in an extension of cellular life span (29-31). A number of small molecules, of which resveratrol, a polyphenol found in red wine and is the most potent, have been reported to mimic calorie restriction and to activate SIRT1, which in turn results in an extension of cellular lifespan through stimulation of SIRT1-dependent deacetylation of p53 (32,33). In this study, we report the chromosomal localization and genomic organization of the murine *Sirt1* gene.

Materials and methods

Identification of the murine Sirt1 cDNA. Homology searches of the EST database at the National Center for Biotechnology Information (NCBI) with the yeast SIR2 protein sequence (GenPept P06700) yielded 10 mRNA sequences of variable length, of which mRNA sequence NM_019812, contained the murine Sirt1 mRNA reference sequence and was used for the identification of the murine *Sirt1* genomic clone.

Identification of BAC genomic clone RP23-390D8. The murine *Sirt1* genomic clone was identified from a murine BAC genomic library (RZPD, Berlin, Germany) after *in silico* screening the *Sirt1* cDNA (GenBank clone NM_019812), which contained the full-length murine *Sirt1* cDNA. BAC clone RP23-390D8 was identified to contain an insert with a size of ~120 kb in the vector pBACe3.6, which included the murine *Sirt1* genomic sequence. BAC genomic DNA was prepared according to published protocols (34) and the murine *Sirt1* insert was confirmed by cycle sequencing (35).

Instrumental methods. Dye terminator cycle sequencing was performed using the ABI PRISM™ BigDye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq™ DNA polymerase (Perkin Elmer, Branchburg, NJ) and analyzed with an ABI PRISM 310 Genetic Analyzer which utilizes the four-color sequencing chemistry.

PCR methods. The Sirt1 sequence was partially sequenced by primer walking on both strands using a direct sequencing strategy (35). Sequencing reactions were performed using 0.6 μg cDNA and 20-30 mer oligonucleotide primers (Thermo Electron, Dreieich, Germany). Sequencing reactions were set up in a volume of 20 μl containing 10 pmol of the sequencing primer, 4 μl BigDye Terminator Cycle Sequencing Ready Reaction Mix (Perkin Elmer, Norwalk, CT), DNA as indicated and ddH_2O to a final volume of 20 μl. The thermal cycling profile for the sequencing of the cDNA-clones was as follows: denaturation at 95˚C for 30 sec, annealing at 50˚C for 15 sec, extension at 60˚C for 4 min (25 cycles), and storage at 4˚C.

Chromosomal localization by fluorescence in situ hybridization (FISH)

Cell culture and chromosome preparation. Standard chromosome preparations were used from an embryonic mouse fibroblast cell line.

Slide preparation. In order to remove excess cytoplasm, slides were treated with pepsin (0.5 mg/ml in 0.01 M HCl, pH 2.0) at 37°C for 40 min. Slides were then washed 10 min in PBSx1 at room temperature followed by an ethanol series (70, 90 and 100%) and air dried.

BAC genomic clone. RP23-390D8, shown to contain the murine Sirt1 gene, was used as a probe.

Probe labeling. The BAC DNA was labeled by a standard nick translation procedure. Digoxigenin (Roche Diagnostics) was used as labeled dUTP at the concentration of 40 μM. Probe length was analyzed on a 1% agarose gel. The probe showed the optimal average length of ~300 bp after nick translation.

Hybridization and probe detection. DNA (~50 ng) was pooled together with 2 μg cot-1 in 10 μl hybridization buffer (50% formamide, 2X SSC, 10% dextransulfate). The DNA was applied to chromosomes fixed on a slide, mounted with a cover slip and sealed with rubber cement. Probe DNA and chromosomes were denatured together at 72°C for 3 min. Hybridization was overnight at 37°C in a wet chamber. After hybridization, the cover slip was carefully removed and the slide was washed in 2X SSC for 8 min. Slides were then incubated at 70°C in 0.4X SSC/0.1% Tween for 1 min. After equilibration in 4X SSC/0.1% Tween for 5 min, the rhodamine-coupled antibody was applied (dilution 1:400). Incubation was for 45 min at 37°C. The slide was then washed twice in 4 X SSC/0.1% Tween for 10 min at 45°C followed by staining in DAPI (4',6-diamidino-2-phenylindole) for 10 min. For microscopy, the slide was mounted in antifade solution (Vectashield).

Microscopy. *In situ* hybridization signals were analyzed on a Zeiss Axioplan II microscope. Each image plain (blue and orange) was recorded separately with a b/w CCD camera. Chromosomes and FISH signals were then displayed in false colors and images merged on the computer. Camera control, image capture and merging were performed with SmartCapture X software (Digital Scientific, Cambridge, UK).

Sequence analysis and computer database searches. DNA sequence analysis was performed using the Heidelberg Unix Sequence Analysis Resources (HUSAR) server hosted by the Biocomputing Service Group at the German Cancer Research Center (DKFZ, Heidelberg) and the UniGene and LocusLink programs at the NCBI. Sequence comparisons were performed with the BLAST algorithm of the GenBank and EMBL databases (36). Protein similarity scores were calculated from fast alignments generated by the method of Wilbur and Lipman with the Clustal W Multiple Alignment Program Version 1.7 and with the BLAST algorithm at NCBI (Table II) (37). Protein motifs were identified online at the Expert Protein Analysis System (ExPASy) proteomics server of the Swiss Institute of Bioinformatics (SIB) with the program Prosite and double-checked using the MotifFinder program hosted by the GenomeNet WWW server at the Institute for Chemical Research, Kyoto University (Japan), but still remain unconfirmed. Potential transcription factor binding sites were identified with the Transfac program, which is part of the GenomeNet Computation Service, hosted by the Bioinformatics Center at the Institute for Chemical Research at the Kyoto University. Sequence similarities were calculated with the GAP software, which considers all possible alignments and gap positions between two sequences and creates a global alignment that maximizes the number of matched residues and minimizes the number and size of gaps on the HUSAR server. Repetitive elements were identified on the Repeat Masker Server at the University of Washington and CpG elements were found with the CPG software hosted by the European Bioinformatics Institute (EMBL outstation) (Figs. 1 and 2).

Phylogenetic analysis. Phylogenetic trees were constructed from the known murine class III histone deacetylase sequences, which were obtained from a protein sequence similarity search with the yeast SIR2 protein using the BLAST 2.0 program at NCBI database (Non-redundant GenBank CDS: translations+ PDB+SwissProt+SPupdate+PIR). Progressive multiple sequence alignments were performed with the Clustal W Multiple Alignment Program Version 1.7. Trees were calculated and drawn with the Puzzle software, which constructs phylogenetic trees from molecular sequence data and which is hosted by the HUSAR server and displayed with the Phylip2tree program. While vertical numbers along branches represent percentage values for boot-strap statistical support, horizontal values indicate the percent divergence figures between two pairs of sequences (Fig. 5).

Results

Identification of cDNAs encoding murine Sirt1. Homology searches of the EST database at NCBI with the yeast SIR2 protein sequence (GenPept P06700) yielded 10 mRNA sequences of variable length: BC152314 (1,814 bp), BC006584 (2,209 bp), AY377984 (3,906 bp), AK144203 (3,743 bp), AK141245 (1,631 bp), AK140330 (686 bp), AK139946 (1,173 bp), AF214646 (3,849 bp), AK213131 (159 bp) and NM_019812 (3,849 bp), of which only AY377984, AF214646 and NM_019812 contained the full length murine Sirt1 mRNA. Clone NM_019812, which contains the murine Sirt1 mRNA reference sequence was then used for the identification of the murine Sirt1 genomic clone. The authenticity of its insert was confirmed by DNA cycle sequencing. Sequences flanking the 5' and 3' ends of the *Sirt1* open reading frame were identified from the *Sirt1* murine genomic clone BAC RP23-390D8. Characterization of the 5' flanking genomic region, which precedes the *Sirt1* open reading frame, revealed a number of putative optimal transcription factor binding sites for NFκB and GATA in addition to a small 393 bp CpG island (pos. -55 to -447). However, their biological relevance needs further investigation. The 3,849 bp murine *Sirt1* mRNA encodes a 737 aa protein with a predicted molecular weight of 80.4 kDa and an isoelectric point of 4.62. In addition, an alternatively spliced shorter 3,765-bp transcript has been reported, which encodes a 698 aa protein with a predictive molecular weight of 76.0 kDa and an isoelectric point of 4.60. This alternatively spliced murine Sirt1 protein lacks exon 2 (Ensembl Transcript ID: ENSMUST00000105442). Fluorescence *in situ* hybridization analysis localized the murine *Sirt1* gene to chromosome 10B4. Translational stop codons in all reading frames precede the murine *Sirt1* open reading frame. The 3' flanking region was shown to contain the eukaryotic polyadenylation consensus signal ATTAAA 1,598 bp downstream of the termination of translation signal TAA (Fig. 1).

Identification and characterization of the murine Sirt1 genomic locus. The murine *Sirt1* genomic clone was obtained from an arrayed murine BAC genomic library from the RZPD German Resource Center for Genome Research (Berlin, Germany) after *in silico* screening with the murine *Sirt1* cDNA (GenBank clone *NM*_019812), which contained the full-length murine *Sirt1* cDNA sequence. BAC clone *RP23-390D8* was identified to contain inserts with an average size of ~120 kb in the 11.6-kb vector pBACe3.6, which included the murine *Sirt1* genomic sequence. BAC genomic DNA was prepared according to published protocols (34) and the *Sirt1* insert was confirmed by cycle sequencing (35). Genomic sequence comparison analyses with the BLAST algorithm helped with the identification of mouse chromosome 10 genomic contig GenBank NC_000076. This sequence is part of the largely finished reference sequence (C57BL/6J) that contains small amounts of WGS and HTGS Draft sequence and was assembled by NCBI in consultation with the Mouse Genome Sequencing Consortium. We used this sequence for the determination of *Sirt1* introns and exon/intron boundaries (Table I). The murine *Sirt1* gene spans a region of 19,910 bp (Fig. 2). Determination of the exon-intron splice junctions established that the gene *Sirt1* is encoded by 9 exons ranging in size from 80 bp (exon 6) to 1,927 bp (exon 9). Within introns 2, 3, 4 and 6 in particular, we identified an accumulation of interspersed repetitive

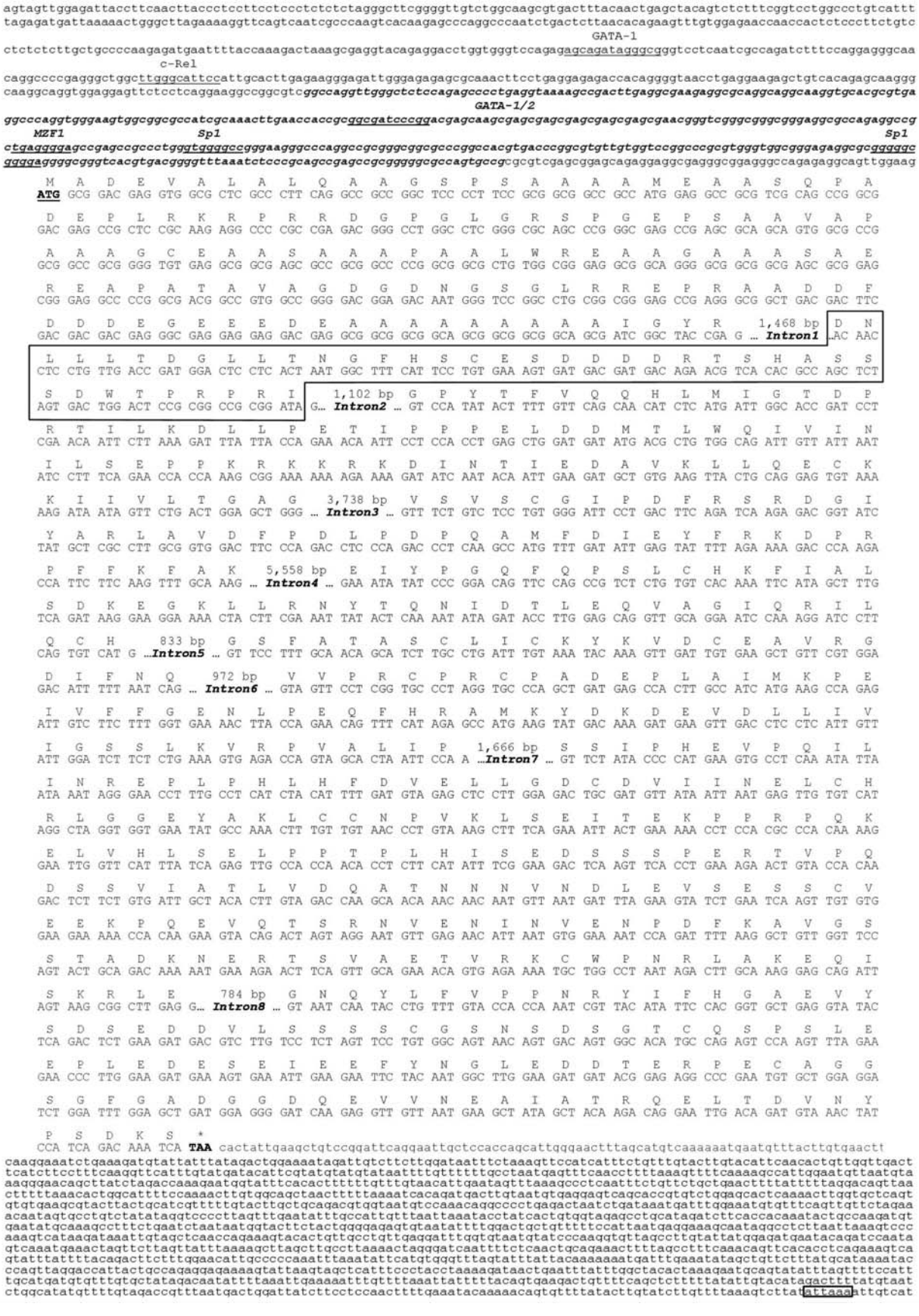

Figure 1. The complete sequence of the murine *Sirt1* cDNA together with the predicted amino acid sequence is shown with the location of each intron with respect to the cDNA sequence. The 3,849 bp murine *Sirt1* mRNA has an open reading frame of 2,214, which yields a 737 aa protein and an untranslated 3' flanking region, which is 1,598 bp (distance from the translational termination codon to the polyadenylation signal). The 2 kb of 5' upstream promoter region are indicated 5' upstream of the translational start codon. Putative transcription factor binding sites are underlined in bold italics. A small CpG island that measures 393 bp in length, is shown in bold lower case. The translational start (ATG) and stop codons (TAA) are underlined, the polyadenylation signal (ATTAAA) is boxed.

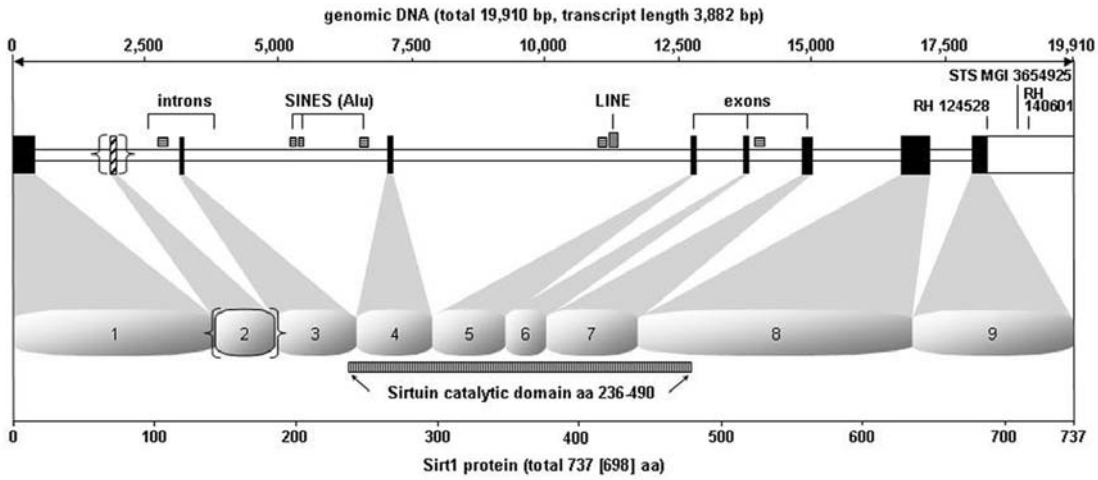

Figure 2. Genomic organization of the murine *Sirt1* gene. The genomic organization of the 19.9 kb *Sirt1* gene, including the relative position of exons and introns, is shown. Repetitive sequences, known as SINEs and LINEs are indicated. The 3' untranslated flanking region of exon 9 contains STS-markers STS-MGI 3654925, RH124528 and RH140601. The sirtuin catalytic domain overlaps the SIRT1 protein region that is encoded by exons 3 through 8.

Table I. Exon/intron splice-junctions of the murine *Sirt1* gene.[a]

Exon no.	Exon size	5'-Splice donor	Intron no.	Intron size	3'-Splice acceptor
1	406	GGCTACCGAG**gt**gagtccgcgc	1	1,468	tctctttgac**ag**ACAACCTCCT
2	117	CCGCGGATAG**gt**atgcttcagg	2	1,102	cccttaatat**ag**GTCCATATAC
3	242	TGGAGCTGGG**gt**atgtaagacg	3	3,738	tgtctgtttc**ag**GTTTCTGTCT
4	153	GTTTGCAAAG**gt**atcacacgtt	4	5,558	tgcattacat**ag**GAAATATATC
5	148	CAGTGTCATG**gt**tagtgacgtc	5	833	tggttgtttt**ag**GTTCCTTTGC
6	80	TTTTAATCAG**gt**aatttaattc	6	972	tgttttgtgc**ag**GTAGTTCCTC
7	187	CTAATTCCAA**gt**aagttgatgt	7	1,666	ccccatttttt**ag**GTTCTATACC
8	552	CGGCTTGAGG**gt**atggaatgcg	8	784	tctgtatttc**ag**GTAATCAATA
9	329 (1,927)				

[a]Exon sequences are given in uppercase and intron sequences are given in lowercase letters. The sizes of the single exons and introns are indicated. Consensus splice donor and splice acceptor sequences are given in bold.

Table II. Sequence identity and similarity among class III sirtuin HDACs in the mouse.[a]

Identity / Similarity	Mouse SIRT1	Mouse SIRT2	Mouse SIRT3	Mouse SIRT4	Mouse SIRT5	Mouse SIRT6	Mouse SIRT7	Yeast SIR2
Mouse SIRT1	-	41	42	31	27	23	23	42
Mouse SIRT2	59	-	51	29	26	27	27	30
Mouse SIRT3	64	66	-	29	30	32	28	38
Mouse SIRT4	48	46	43	-	29	27	27	28
Mouse SIRT5	41	44	45	48	-	23	23	28
Mouse SIRT6	40	42	45	42	38	-	41	24
Mouse SIRT7	41	45	43	43	37	55	-	23
Yeast SIR2	59	48	53	46	42	40	40	-

[a]The indicated numbers represent the percentage of sequence identity and similarity from pairwise sequence comparisons.

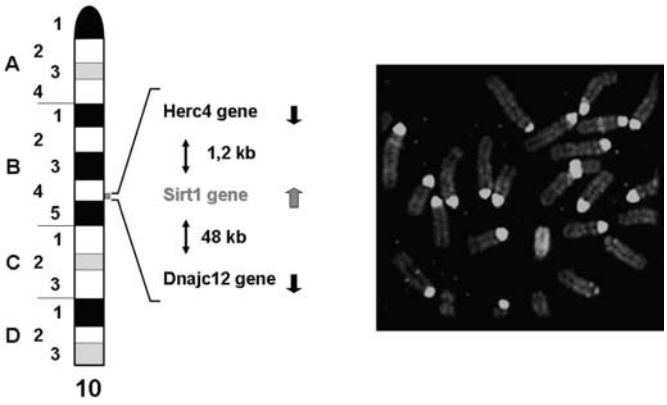

Figure 3. Chromosomal mapping of the murine *Sirt1* gene. Right panel: fluorescence *in situ* hybridization of BAC clone RP23-390D8 to mouse chromosome 10B4. Left panel: chromosome 10 idiogram illustrates the chromosomal position of the *Sirt1* gene, which is closely neighbored by the *Herc4* and *Dnajc12* genes.

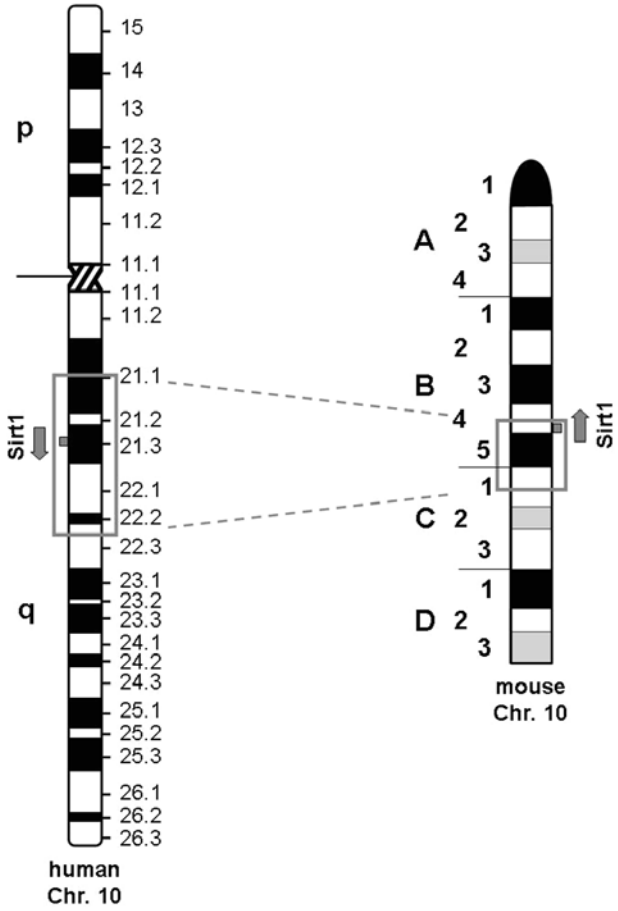

Figure 4. The *Sirt1* gene is located within autosomal gene synteny groups in mice and humans (mouse *Sirt1* is located on chromosome 10B4 and human *Sirt1* is located on chromosome 10q21.3) and is therefore syntenically conserved.

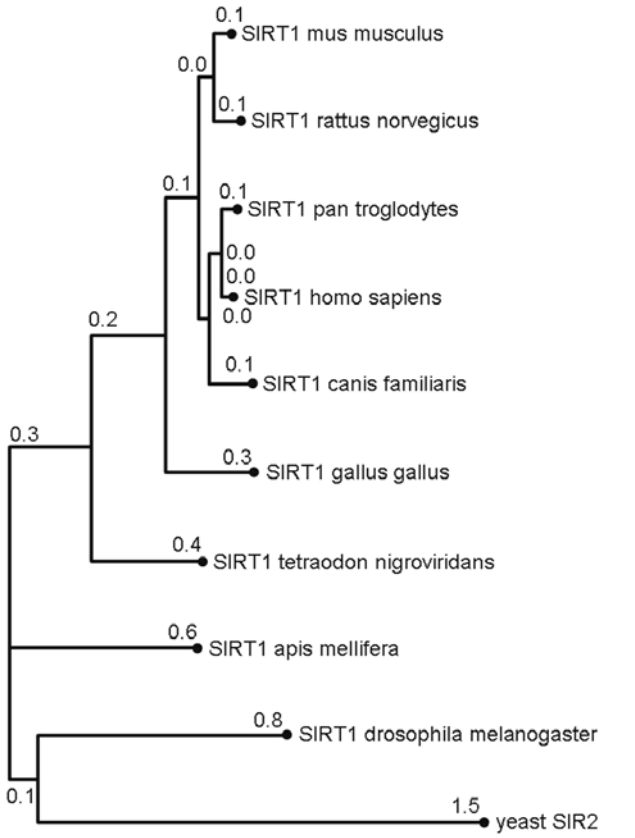

Figure 5. Consensus evolutionary tree on the basis of an alignment of SIRT1 from different species together with their common ancestor protein, yeast SIR2.

elements, short interspersed nuclear elements (SINEs) and long interspersed nuclear elements (LINEs) (Fig. 2). Additionally, we identified STS-marker RH124528 within the translated proportion of murine Sirt1 exon 9 and two internal STS-markers MGI3654925 and RH140601 within the untranslated portion of exon 9 between the *Sirt1* translational termination signal (TAG) and the polyadenylation consensus signal ATTAAA. The sirtuin catalytic domain, which is highly conserved in all members of mammalian sirtuins that have been described so far as well as in their Sir2 yeast ancestor protein, is found between amino acid residues 236 and 490, i.e. within exons 3 and 8 of the protein (Fig. 2).

Murine Sirt1 is a single copy gene. Both sequencing and results obtained by electronic PCR of BAC clone RP23-390D8 identified STS markers STS-MGI 3654925, RH124528 and RH140601 within exon 9 of the *Sirt1* genomic sequence (Fig. 2). Our fluorescence *in situ* hybridization studies localized *Sirt1* to chromosome 10B4. These data, together with the results obtained by electronic PCR and the already known location of the STS markers listed above, indicated one single site of hybridization of *Sirt1* on murine metaphase chromosomes and its specific localization on chromosome 10B4 (Fig. 3), Therefore, it is located within autosomal gene synteny groups in mice and humans (Fig. 4).

Sirt1 expression analyses. *In silico* expression profile analyses was performed with the UniGene EST profile viewer, hosted by the NCBI homepage and suggested the strongest expression of murine *Sirt1* in the epididymis, followed by vaginal tissue, the nasopharynx, the prostate, the thymus,

pituitary gland and the ovary on the basis of an analysis of EST counts.

Phylogenetic analyses and pairwise sequence comparisons. We screened the expressed sequence tag database (NCBI) with the yeast Sir2 protein sequence and identified the SIRT1 protein sequences in various different species. A consensus evolutionary tree was obtained using an alignment of yeast SIR2 with a selection of different mammalian and non-mammalian SIRT1 homologs (Fig. 5). The accession numbers of the sequences that were used in this phylogenetic analysis were as follows: *Apis mellifera* SIRT1 (GenPept XP_395386), *Canis canis* SIRT1 (GenPept XP_546130), *Drosophila melanogaster* SIRT1 (GenPept NP_477351), *Gallus gallus* SIRT1 (GenPept NP_001004767), *Mus musculus* SIRT1 (GenPept Q923E4), *Pan troglodytes* SIRT1 (GenPept XP_521490), *Rattus norvegicus* SIRT1 (GenPept XP_228146), *Tetraodon nigroviridans* SIRT1 (GenPept CAG04727), *Homo sapiens* SIRT1 (GenPept AAD40849), and yeast Sir2 (GenPept P06700).

Discussion

The mammalian family of yeast SIR2 protein homologs is comprised of seven proteins (SIRT1-7), located in different cellular compartments, modify multiple substrates and affect multiple cellular processes such as the response to stress, genome maintenance, metabolism and is an important mediator of organismal longevity through a number of different mechanisms such as the induction of cell cycle arrest, resistance to oxidative stress and the inhibition of apoptosis (42,47). The sirtuins contain a conserved core domain, and for some of the family members, additional N- or C-terminal elongations. While knowledge is rapidly accumulating for the NAD^+-dependent deacetylase SIRT1, far less is known about the other sirtuins (11). Mammalian SIRT1 is located in the cell nucleus (26,27). Its physiological effects are mediated by SIRT1-dependent deacetylation of histones and non-histone proteins, including p53 (26,27), the forkhead transcription factor FOXO3A (28), PML (38), BCL6 (48), TAF_I68 (39), HES1 (40), NFκB, PGC-1 and CTIP2 (41,42). SIRT1 is essential during embryonic development (43,44) and tissue differentiation (45). While the mammalian proteins SIRT4 and 6 primarily mediate ADP-ribosylation, most of the mammalian sirtuins (SIRT1, 2, 3, and 5) catalyze the NAD^+-dependent deacetylation of proteins. Within the family of mammalian sirtuins, SIRT1 is the one with the closest homology to yeast Sir2.

To date, *Sirt1* knockout mice have been generated by two different groups and both groups found the *Sirt1* knockout mice to be viable. However, these mice had a lower birth ratio, were smaller than the wild-type animals and had a lower survival rate at birth (43,44). One apparent developmental defect were eye deformities and an eyelid opening delay for several months after birth (44). Additionally, the lung, the pancreas and the heart were also affected in knockout mice (43,44). In accordance with our analyses, the *Sirt1* transcript is widely expressed in many tissues with a strong expression in the testis and epididymis, followed by vaginal tissue, the nasopharynx, the prostate, the thymus, pituitary gland and the ovary. Notably, both sexes of the null animals were reported to be sterile with female animals having small ovaries and a thin uterine wall (43) and males having severely reduced numbers of mature sperm, which were malformed and immotile (43). Also, the levels of p53 acetylation were reported much higher in *Sirt1* knockout mice (44).

In this study, we report the cloning, characterization and mapping of murine *Sirt1* on the genomic level. *Sirt1* is a single-copy gene in mice that spans a region of ~19.9 kb. It is composed of 9 exons (Table I) ranging in size from 80 bp (exon 6) to 1,927 bp (exon 9) and reveals an accumulation of interspersed repetitive elements within introns 2, 3, 4 and 6 in particular, which consist of *Alu* or *Kpn*I and *Bam*H1 repeats as representative examples of SINEs (*Alu* repeats) and LINEs (*KpnI* and *BamH1* repeats) (46). The histone deacetylase catalytic domain is highly conserved within all members of mammalian HDACs that have been described so far and is found between amino acid residues 236 and 490, i.e. within exons 3 and 8 of the protein (Fig. 2). The 5' upstream *Sirt1* promoter region was found to contain a number of NFκB and GATA transcription factor binding sites in addition to a small 393 bp CpG island (pos. -55 to -447) (Fig. 1). The murine *Sirt1* mRNA encodes a 737 aa protein with a predictive molecular weight of 80.4 kDa. Fluorescence *in situ* hybridization analysis in conjunction with electronic PCR localized the murine *Sirt1* gene to the sub-band of chromosome 10B4 (Fig. 3).

The sirtuin proteins are known to be critical in the regulation of life span. Since cancer is an age-related disease, the sirtuins are based on their key role in the stress response to genomic damage, gaining increasing importance as molecular targets in the prevention and treatment of cancer. Even though the sirtuins ensure that damaged DNA is not propagated and accumulated, there is also increasing evidence that they are promoting growth in some types of cancer, which needs however to be further investigated.

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