Cloning, chromosomal characterization and FISH mapping of the NAD$^+$-dependent histone deacetylase gene sirtuin 5 in the mouse

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Abstract. Sirtuin 5 (SIRT5) is a nicotinamide adenine dinucleotide (NAD$^+$)-dependent deacetylase, belonging to the silent information regulator 2 (Sir2) family of sirtuin histone deacetylases (sirtuins). The yeast Sir2 protein and its mammalian derivatives are important in epigenetic gene silencing, DNA repair and recombination, cell cycle, microtubule organization and in the regulation of aging. In mammals, 7 sirtuin isoforms have been identified to date of which three (SIRT3, SIRT4 and SIRT5) are localized in the mitochondria, which serve as the center of energy management and the initiation of cellular apoptosis. In the study presented herein, we report the genomic organization and chromosomal localization of the murine *sirt5* gene. We have isolated and characterized the murine *sirt5* genomic sequence, which spans a region of 24,449 bp and which has one single genomic locus. The murine *sirt5* gene consists of 8 exons and encodes a 310-aa protein with a predictive molecular weight of 34.1 kDa and an isoelectric point of 8.90. For the murine *sirt5* gene only one single genomic locus has been identified. The gene has been localized to mouse chromosome 13A4 and is flanked by STS-marker 164522 (synonymous WI MRC-RH: 506859).

Introduction

Sirtuins (SIRT1-SIRT7) are NAD$^+$-dependent hydrolases (1,2). Derivatives of the yeast SIR2 histone deacetylase have a common catalytic domain, which is highly conserved in multiple organisms ranging from bacteria to humans. The sirtuin domain is composed of two distinct motifs binding NAD$^+$ and the acetyl-lysine substrate, respectively (3,4). They catalyse the deacetylation of acetylated lysine residues of histone substrates and act on non-histone substrates, e.g., several transcription factors such as the p53 tumor suppressor protein (5), the cytoskeletal protein α-tubulin (6) or the acetyl-CoA synthetase (7). Besides the deacetylation reaction, some sirtuins show ADP ribosyltransferase activity (8,9).

A dysregulation of the tightly regulated equilibrium of acetylation and deacetylation plays an important role both, in the pathogenesis and in the suppression of cancer (10). Histone acetylation modifiers are therefore becoming more important as potential targets in the treatment of cancer. Relaxation of the chromatin fiber facilitates transcription and is controlled by two competing enzymatic activities, histone acetyltransferases (HATs) and histone deacetylases (HDACs), which modify the acetylation state of histone proteins and other promoter-bound transcription factors. While HATs, which are frequently part of multisubunit coactivator complexes, induce the relaxation of chromatin structure and transcriptional activation, HDACs tend to associate with multisubunit corepressor complexes, which result in chromatin condensation and transcriptional repression of specific target genes. HAT and HDAC enzymatic activities are involved in the generation and in the suppression of cancer. Some of the genes encoding these enzymes have been shown to be rearranged in the context of chromosomal translocations in human acute leukemias and solid tumors, where fusions of regulatory and coding regions of a variety of transcription factor genes result in completely new gene products, which may interfere with regulatory cascades that control cell growth and differentiation (10). On the other hand, some histone acetylation modifying enzymes have been located within chromosomal regions being particularly prone to chromosomal breaks. In such cases, gains and losses of chromosomal material may affect the availability of functionally active HATs and HDACs, which in turn disturbs the tightly controlled equilibrium of histone acetylation (11).

Sirtuins are important for various biological processes, including cellular development, heterochromatin formation, gene/transcriptional silencing (12-16), DNA repair, genome stability and cellular processes such as the response to stress,

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adipogenesis and metabolism and are 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 (17). Sirtuins connect aging, cancer, and diet and thus are potential molecular targets for the development of pharmaceuticals to treat human malignant, metabolic and neurological diseases (18).

Based on structural and functional similarities, mammalian histone deacetylases are classified into four categories, of which three contain non-sirtuin HDACs comprising the yeast RPD3 homologs (class I HDACs), the HDA1 mammalian homologs (class II HDACs) and HDAC11-related enzymes (class IV HDACs), while one category consists of sirtuin histone deacetylases (class III HDACs), being homologs of the yeast Sir2 protein (19). In contrast to SIRT1, the SIRT5 protein is still poorly investigated (6,7,19,20).

Materials and methods

Identification of the murine Sirt5 cDNA. Homology searches of the EST database at NCBI (National Center for Biotechnology Information) with the yeast SIR2 protein sequence (GenPept P06700) yielded 4 mRNA sequences of variable length, of which mRNA sequence NM_178848.3, which in the meantime is referred to as the NCBI reference sequence for Sirt5, contained the full length murine Sirt5 mRNA which was then used for the identification of the murine Sirt5 genomic clone.

Identification of BAC genomic clone RP24-62L21. The murine Sirt5 genomic clone was identified from a murine BAC genomic library (RZPD, Berlin, Germany) after *in silico* screening with the Sirt5 cDNA (GenBank clone NM_178848.3), which was shown to contain the full-length murine Sirt5 cDNA. BAC clone *RP24-62L21* was identified to contain an insert with a size of ~120 kb in the vector pBACe3.6, which included the murine Sirt5 genomic sequence. BAC genomic DNA was prepared according to published protocols (21) and the murine Sirt5 insert was confirmed by cycle sequencing (22).

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 *sirt5* sequence was partially sequenced by primer walking on both strands using a direct sequencing strategy (22). Sequencing reactions were performed using 0.6 μg cDNA and 20-30mer oligonucleotide primers (Thermo Electon, 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 added up 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 a mouse embryonic fibroblast cell line.

Slide preparation. In order to remove excess of 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 1X PBS at room temperature followed by an ethanol series (70, 90 and 100%) and air-dried. *BAC genomic clone RP24-62L21*, which was shown to contain the murine Sirt5 gene, was used as a probe.

Probe labeling. The BAC DNA was labelled by a standard nick translation procedure. Digoxigenin (Roche Diagnostics) was used as labelled 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% dextran sulfate). 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 of 1:400). Incubation was for 45 min at 37˚C. The slide was then washed twice in 4X 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 done with SmartCapture X software (Digital Scientific, Cambridge, UK).

Sequence analysis and computer database searches. DNA sequence analysis was performed using the HUSAR (Heidelberg Unix Sequence Analysis Resources) server hosted by the Biocomputing Service Group at the German Cancer Research Center (Heidelberg, Germany) and the UniGene and LocusLink programs at the National Center for Biotechnology Information (NCBI). Sequence comparisons were performed with the BLAST algorithm of the GenBank and EMBL databases (23). 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 I) (25). Protein motifs were identified online at the ExPASy (Expert Protein Analysis System) 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 Institute for Chemical Research, Kyoto University (Japan), but still remain to be experimentally

Table I. Sequence identity and similarity among class III sirtuin-HDACs.[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 (23).

confirmed. Potential transcription factor binding sites were identified with the TRANSFAC program, which is part of the GenomeNet Computation Service, which is 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 (26). 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).

Results

Identification of cDNAs encoding murine Sirt5. Homology searches of the EST database at NCBI (National Center for Biotechnology Information) with the yeast SIR2 protein sequence (GenPept P06700) yielded 4 mRNA sequences of variable length: AK002609.1 (1,397 bp), AK005346.1 (1,381 bp), BC031770.1 (1,403 bp), BC087898.1 (1,547 bp) and NM_178848.3 (1,369 bp). NM_178848.3, which is the sirt5 reference sequence contained the full length murine Sirt5 mRNA sequence, which was then used for the identification of the murine Sirt5 genomic clone. The authenticity of its insert was confirmed by DNA cycle sequencing. Sequences flanking the 5'- and 3'-ends of the *Sirt5* open reading frame were identified from the *Sirt5* murine genomic clone BAC *RP24-62L21*. Characterization of the 5'-flanking genomic region, which precedes the *Sirt5* open reading frame, revealed a number of putative optimal transcription factor binding sites for GATA, Evi-1, C/EBPb, AP-1, SRY, MZF1 and CdxA (Fig. 1). However, their biological relevance still awaits to be investigated experimentally. Islands of unusual CG composition were not observed. The 24,449-bp murine *Sirt5* gene encodes a 310-aa protein (Fig. 2) with a predictive molecular weight of 34.1 kDa and an isoelectric point of 8.90. Fluorescence *in situ* hybridization analysis localized the murine *Sirt5* gene to mouse chromosome 13A4 (Fig. 3). Translational stop codons in all reading frames precede the human *sirt5* open reading frame. The 3'-flanking region was shown to contain the eukaryotic polyadenylation consensus signal ATTAAA 295 bp downstream of the termination of translation signal TAA (Fig. 1) (27).

Identification and characterization of the murine Sirt5 genomic locus. The murine *Sirt5* 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 *Sirt5* cDNA (GenBank clone NM_178848.3), which was shown to contain the full-length murine *Sirt5* cDNA sequence. BAC clone *RP24-62L21* was identified to contain inserts with an average size of ~120 kb in the 11.6-kb vector pBACe3.6, which included the murine *Sirt5* genomic sequence. BAC genomic DNA was prepared according to published protocols (22) and the *Sirt5* insert was confirmed by cycle sequencing (23). Genomic sequence comparison analyses with the BLAST

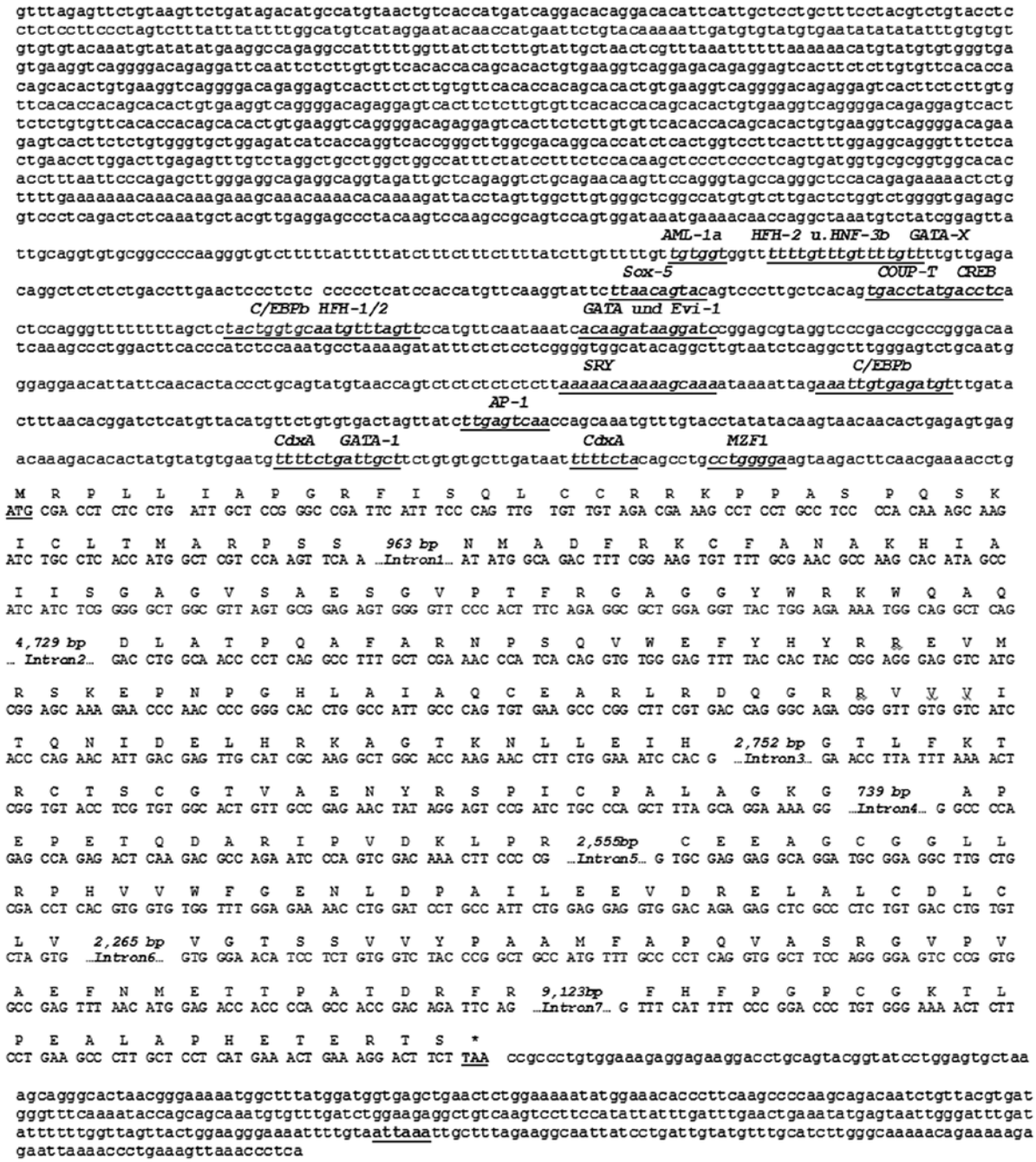

Figure 1. The complete sequence of *Sirt5* cDNA together with the predicted amino acid sequence is shown with the location of each intron with respect to the cDNA sequence. The 24,449-bp murine *Sirt5* mRNA has an open reading frame of 930 bp which yields a 310-aa protein and an untranslated 3'-flanking region (exon 8), which was shown to contain the eukaryotic polyadenylation consensus signal ATTAAA 295 bp downstream of the termination of translation signal TAA. Two kb of the 5'-upstream promoter region are indicated 5'-upstream of the translational start codon. Putative transcription factor binding sites are underlined in bold italics. The translational start (ATG), then stop codon (TAA) and the polyadenylation signal (ATTAAA) are underlined.

algorithm helped us with the identification of mouse chromosome 13 genomic contig GenBank NC_000079. 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 have used this sequence for the determination of *Sirt5* introns and exon/intron boundaries (Table II). The murine *Sirt5* gene spans a region of 24,449 bp. Determination of the exon-intron splice junctions established that the gene *Sirt5* is encoded by 8 exons ranging in size from 54 bp (exon 5) to 466 bp (exon 8). Within introns 5, 6 and 7 in particular, we identified an accumulation of interspersed repetitive elements, SINEs (short interspersed nuclear elements) and LINEs (long interspersed nuclear elements). Additionally, we have identified STS-marker 164522 (synonymous WI MRC-RH: 506859) within the untranslated proportion of murine Sirt5 exon 8 between the *Sirt5* translational termination signal (TAA) and the polyadenylation consensus signal ATTAAA. The sirtuin catalytic domain, which is highly conserved in all members

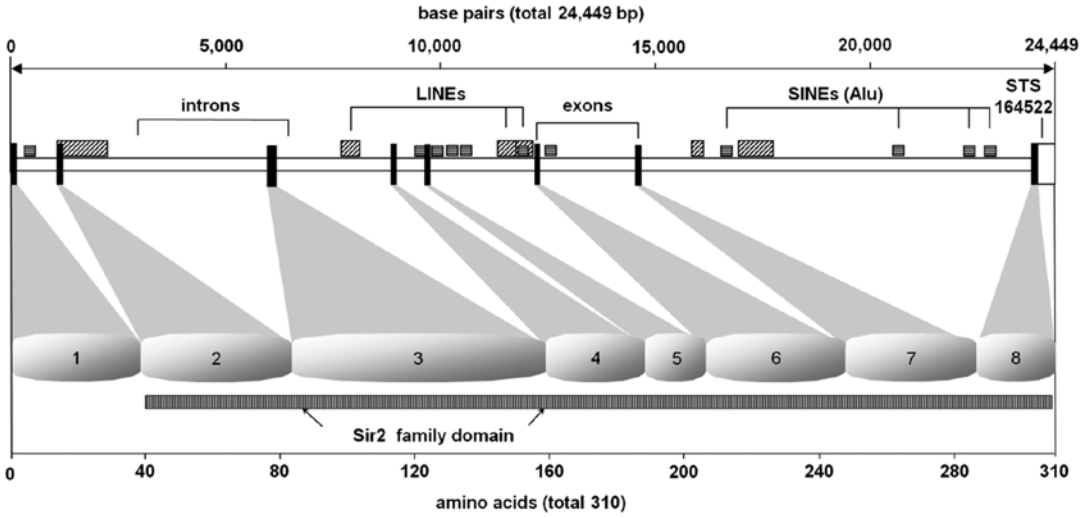

Figure 2. Genomic organization of the murine *Sirt5* gene. The genomic organization of murine *Sirt5* gene, which includes the relative position of exons and introns is shown. Repetitive sequences, known as short and long interspersed nuclear elements (SINEs and LINEs) are indicated.

Table II. Exon/intron splice-junctions of the murine *Sirt5* gene.

Exon no.	Exon size	5'-Splice donor	Intron no.	Intron size	3'-Splice acceptor
1	115	CATGGCTCGTgtaagtcatctg	1	963	ttttctgttt**ag**ATATGGCAGA
2	134	GCAGGCTCAGgttagtaacgct	2	4729	ctctcccctc**ag**GACCTGGCAA
3	226	GAAATCCACGgtgaggagaacg	3	2752	ctttttttc**ag**GAACCTTATT
4	88	CAGGAAAAGGgtaagtatagca	4	739	tatttgctcc**ag**GGCCCCAGAG
5	54	AACTTCCCCGgtaggtaaaaca	5	2555	atgctcttcc**ag**GTGCGAGGAG
6	124	GTGTCTAGTGgtaagtcacatg	6	2265	cttgctttgt**ag**GTGGGAACAT
7	116	ACAGATTCAGgtacagggacaa	7	9123	tcttgtgttt**ag**GTTTCATTTT
8	466				

Exon sequences are given in upper case and intron sequences are given in lower case letters. The sizes of the single exons and introns are indicated. Consensus splice donor and splice acceptor sequences are given in bold.

of mammalian sirtuins that have been described so far as well as in their Sir2 yeast ancestor protein, is found between amino acid residues 41 and 309, i.e., within exons 1 and 8 of the protein (Fig. 2).

Murine Sirt5 is a single copy gene. Both sequencing and results obtained by electronic PCR of BAC clone *RP24-62L21* identified STS-marker 164522 (synonymous WI MRC-RH: 506859) within the untranslated proportion of murine *sirt5* exon 8 genomic sequence (Fig. 2). Our fluorescence *in situ* hybridization studies localized BAC clone *RP24-62L21*, which contains the murine *sirt5* genomic sequence to the chromosome 13A4/5 border region. However, the more precise high-resolution analyses assigned murine *sirt5* to mouse chromosome 13A4. Taken together, the results obtained by FISH, electronic PCR and the already known location of the STS marker listed above, indicated one single site of hybridization of *sirt5* on mouse metaphase chromosomes and its specific localization on chromosome 13A4 (Fig. 3).

Phylogenetic analyses and pairwise sequence comparisons. Using the consensus murine sirtuin (class III deacetylase) protein sequences together with the class I, II and IV murine histone deacetylase protein sequences, a consensus evolutionary tree was calculated (Fig. 4), which reveals the evolutionary position of the murine SIRT5 protein. The accession numbers of the sequences that have been used in this phylogenetic analysis were as follows: Yeast Sir2 (GenPept P06700), Mus musculus SIRT3 (GenPept CAJ18608.1), Mus musculus SIRT2 (GenPept AAH21439.1), Mus musculus SIRT1 (GenPept Q923E4), Mus musculus SIRT4 (GenPept XP_993153), Mus musculus SIRT7 (GenPept NP_694696.2), Mus musculus SIRT6 (GenPept AAH52763.1), Mus musculus SIRT5 (GenPept AAH31770.1), Mus musculus HDAC1 (GenPept CAQ 51569.1), Mus musculus HDAC2 (GenPept AAI38518.1), Mus musculus HDAC3 (GenPept AAF36425.1), Mus Musculus HDAC4 (GenPept AAH66052.1), Mus musculus HDAC5 (GenPept CAX15947.1), Mus musculus HDAC6 (GenPept AAH41105.1), Mus musculus HDAC7 (GenPept AAH57332.1),

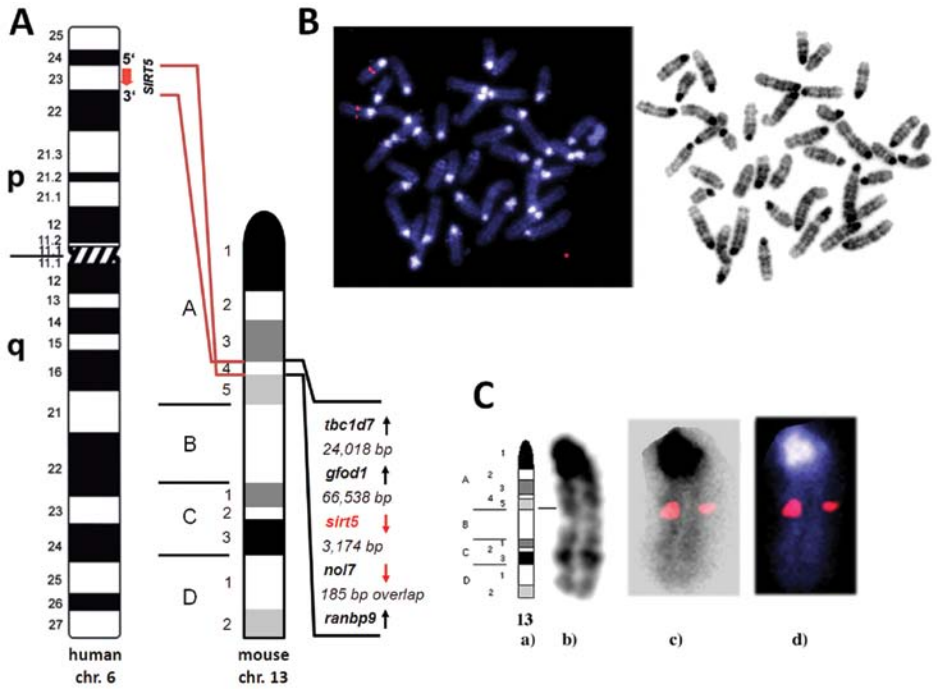

Figure 3. Chromosomal mapping of the murine *Sirt5* gene. (A) Chromosome 13 idiogram according to the International System for Cytogenetic Nomenclature (ISCN 1995), which illustrates the chromosomal position of BAC clone *RP24-62L21*. Neighboring genes are also indicated. The chromosomal orientation of *Sirt5* is shown (arrow). The syntenic conservation between murine sirt5 on mouse chromosome 13A4 and human chromosome 6p23 (19) is indicated. (B) Fluorescence *in situ* hybridization of BAC clone *RP24-62L21* localized the murine *Sirt5* gene to mouse chromosome 13A4. (C) From left to right, next to the chromosome 13 idiogram, images of a DAPI-stained chromosome 13, together with the same chromosome carrying the BAC hybridization signal are illustrated.

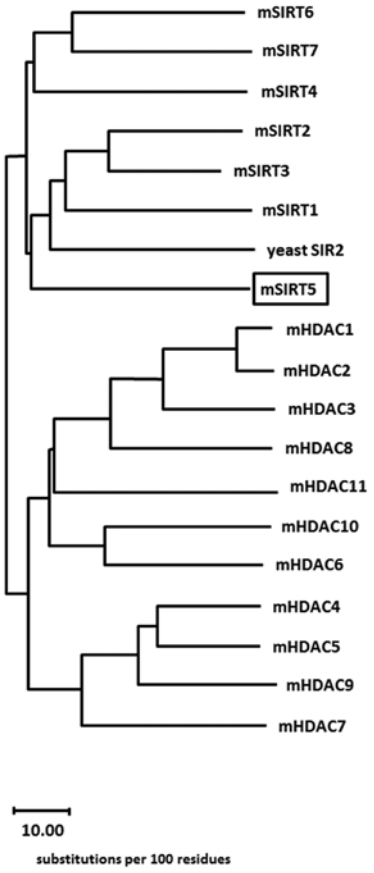

Figure 4. Consensus evolutionary tree on the basis of an alignment of murine SIRT5 in relation to the other murine non-sirtuin HDACs and sirtuin deacetylases (24).

Mus musculus HDAC8 (GenPept AAH 61257.1), Mus musculus HDAC9 (GenPept AAH98187.1), Mus musculus HDAC10 (GenPept AAH64018.1) and Mus musculus HDAC11 (GenPept AAH16208.1). In Table I the sequence identity and similarity among class III sirtuin-HDACs is demonstrated. The indicated numbers represent the percentage of sequence identity and similarity from pairwise sequence comparisons.

Discussion

Sirtuins show different subcellular distribution, different substrate specificity and cellular function. In the nucleus, SIRT1 functions (31) as a transcriptional repressor via histone deacetylation. SIRT1 also regulates transcription by modifying the acetylation levels of transcription factors, e.g., MyoD, FOXO, p53, and NF-κB (5,32-36). In the cytoplasm the SIRT2 protein associates with microtubules and deacetylates α-tubulin lysine 40 (6). SIRT6 regulates telomeric chromatin and is a histone H3K9 deacetylase (37). In the nucleolus, SIRT7 functions as a positive regulator of RNA polymerase I transcription (38).

Three mammalian sirtuins (Sirt3, 4, and 5) are localized in the mitochondria, the center of energy metabolism and apoptosis initiation (39). Electrons pass through electron transport complexes (I-IV), generating a proton gradient that is used to drive ATP synthase to generate ATP. SIRT3 binds to complex I, binds and deacetylates acetyl-CoA synthetase 2 (AceCS2) and glutamate dehydrogenase (GDH), activating their enzymatic activities. SIRT4 binds and represses GDH activity via ADP-ribosylation (40).

The exact localization of Sirt5 within the mitochondria differs. In transfected COS7 cells, murine FLAG-tagged Sirt5 was solely found in the mitochondrial intermembrane space (41), whereas endogenous murine Sirt5 protein was exclusively found in the mitochondrial matrix (42) in liver cells (31,45). SIRT5 may localize to the mitochondrial intermembrane space after overexpression or after mitochondrial import *in vitro* (31,41,43). It is therefore discussed that the localization of SIRT5 may in fact depend on the type of underlying stimulus, whether SIRT5 is translocated predominantly into the mitochondrial intermembrane space or to the mitochondrial matrix (40).

Because of its localization in mitochondria, SIRT5 deacetylates and activates carbamoyl phosphate synthetase 1 (CPS1), the rate-limiting step of the urea cycle (40) and urate oxidase in murine liver mitochondria (46). SIRT5 exhibits deacetylase activity against acetylated cytochrome C, a conserved mitochondrial intermembrane space protein (31,43) and regulates various mitochondrial metabolic pathways together with SIRT3 and SIRT4 (31). In contrast to Sirt3, Sirt5 does not deacetylate any of the mitochondrial matrix proteins tested (43). SIRT5 exhibits weak but detectable deacetylase activity against acetylated histone H4 (31,47), as well as chemically acetylated histones or acetylated BSA (2,31). Whether Sirt5 has an additional function in the regulation of apoptosis, remains speculative (44), but there is a physiological role of Sirt5 in the regulation of cellular metabolism and cellular senescence (2,39).

The role of Sirt5 in the pathogenesis of human diseases is currently being discussed since repetitive elements in the gene structure of Sirt5 may partly induce genomic instability and thus malignant transformation (44). Besides, Sirt5 could contribute to liver damage as a consequence of chronic alcohol consumption (48), since alcohol exposure may induce posttranslational modification such as hyperacetylation of numerous proteins including p53, ACS2 and tubulin (44,49). Since hepatic expression levels of Sirt5, but not Sirt3, are significantly diminished upon exposure to ethanol (48); this suggests that Sirt5 may contribute to the hyperacetylation of ethanol-dependent proteins (44). Also, the depletion of the Sirtuin cosubstrate NAD^+ is known to accompany alcohol exposure and the highly reactive intermediates that are associated with alcohol metabolism indicate an additional, more direct mechanism how Sirt5 activity may be affected. The emerging role of Sirt5 in energy and amino acid metabolism in liver mitochondria suggests that changes of its activity may indeed contribute to the pathology of alcohol associated organ disease, but details will have to await further studies (44).

In the study presented herein, we report the cloning, characterization and mapping of murine *sirt5* on the genomic level. Murine *Sirt5* is a single-copy gene that spans a region of 24,449 bp. It is composed of 8 exons ranging in size from 54 bp (exon 5) to 466 bp (exon 8) (Table II). Particularly within introns 5, 6 and 7 we identified an accumulation of interspersed repetitive elements, which consist of *Alu* or *Kpn*I and *Bam*H1 repeats as representative examples of short and long interspersed nuclear elements, known as SINEs (*Alu* repeats) and LINEs (*Kpn*I and *Bam*H1 repeats) (50). Additionally, we identified an internal STS-marker 164522 (synonymous WI MRC-RH: 506859), within the untranslated proportion of *sirt5* exon 8 between the *Sirt5* translational termination signal (TAA) 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 41 and 309, i.e., within exons 1 and 8 of the protein. The 1,369-bp human *Sirt5* mRNA (GenBank NM_178848) has an open reading frame of 930 bp that encodes 310-aa protein with a predictive molecular weight of 34.1 kDa and an isoelectric point of 8.90. Characterization of the 5'-flanking genomic region, which precedes the *Sirt5* open reading frame, revealed a TATA- and CCAAT-box less promoter that contained a number of putative optimal transcription factor binding sites for GATA, Evi-1, C/EBPb, AP-1, SRY, MZF1 and CdxA. However, their biological relevance awaits investigation experimentally. Islands of unusual CG composition were not observed. The sirtuin deacetylase catalytic domain is highly conserved within all members of mammalian sirtuins described so far and located within exons 1-8 (Fig. 2).

Fluorescence *in situ* hybridization analysis in conjunction with electronic PCR localized the murine *Sirt5* gene to chromosome 13A4 (Fig. 3); a genomic area, which shows syntenic conservation with human chromosome 6p23, the human Sirt5 genomic locus, a region which has been found to be involved in numerous chromosomal abnormalities associated with malignant disease, especially as part of both balanced and unbalanced chromosomal abnormalities in acute myeloid leukemia in accordance with data that have been retrieved from the Cancer Genome Anatomy Project (CGAP) database at the National Cancer Institute (19,51). The sirt5 gene is being transcribed towards the downstream distal end of chromosome 13 and is closely neighboured by the *nol7* (nucleolar protein 7) gene (3,174 bp downstream) and the gfod1 (glucose-fructose oxidoreductase domain containing 1) gene (66,538 bp upstream).

It is currently not clear whether and to what extent chromosomal abnormalities involving the human chromosomal locus 6p23 or the murine chromosomal locus 13A4 affect SIRT5-mediated functional effects. It is, however, obvious that several genes encoding sirtuin proteins are located within chromosomal regions that are particularly prone to chromosomal alterations. In such cases, gains and losses of chromosomal material may influence the availability of functionally active sirtuin proteins and thus the tightly controlled intracellular equilibrium of protein acetylation and/or ADP ribosylation, respectively (11). The murine SIRT5 gene is localized at mouse chromosome 13A4, a chromosomal region that has been reported to be associated with malignant disease such as pancreatic cancer in humans (19,31,40). SIRT5 is ubiquitously expressed in various tissues, with relatively high Sirt5 expression level in the heart, skeletal muscle, brain, liver, testis and kidney (7,19,28,29,31,39,40,42). Sirtuin 5 (SIRT5) is distributed widely in all prokaryotes either bacteria or archaea (52). The wide tissue distribution for Sirt5 expression, with significant levels in all tissues tested, might indicate that Sirt5 fulfills general functions needed in all tissues (44). In humans, analyses of expressed sequence tag databases indicated that Sirt5 is predominantly expressed in lymphoblasts, beside heart muscle cells and thymus, leading to the suggestion that

chromosomal breaks in SIRT5 might contribute to myeloid leukemia (19). However, a direct involvement of SIRT5 and its repetitive elements in malignant diseases remains to be shown. The further functional characterization of murine SIRT5 may help to elucidate its potential role, and possibly becoming an exciting endeavor.

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