SPANDIDOS PUBLICATIONS

Cloning, chromosomal characterization and mapping of the NAD-dependent histone deacetylases gene sirtuin 1

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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 sirtuin histone deacetylases (HDACs). The yeast Sir2 protein and its mammalian derivatives play a central role in epigenetic gene silencing, DNA repair and recombination, cell-cycle, microtubule organization, and in the regulation of aging. We have isolated and characterized the human *Sirt1* genomic sequence, which spans a region of 33,660 bp and which 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 2,120 bp (exon 9). Characterization of the 5' flanking genomic region, which precedes the *Sirt1* open reading frame, revealed a CCAAT-box and a number of NF-κB and GATA transcription factor binding sites in addition to a small 350 bp CpG island. The 4,107 bp human *Sirt1* mRNA has an open reading frame of 2,244 bp and encodes a 747 aa protein with a predictive molecular weight of 81.7 kDa and an isoelectric point of 4.55. Fluorescence *in situ* hybridization analysis localized the human *Sirt1* gene to chromosome 10q21.3.

Introduction

Based on structural and functional similarities, mammalian histone deacetylases are grouped into four categories consisting of three classes of non-sirtuin HDACs comprising the yeast histone deacetylase RPD3 homologs (class I HDACs), class II HDACs, which share a significant degree of homology with the yeast histone deacetylase HDA1, the most recently described class IV HDACs, which comprises HDAC11-related enzymes and one class of sirtuin histone deacetylases (class III HDACs), which are homologs to the yeast Sir2 protein with SIRT1 being the most highly related to *S. cerevisiae* Sir2. The currently known seven human sirtuins have been further subgrouped into four distinct phylogenetic classes: SIRT1, SIRT2, SIRT3 (subclass 1), SIRT4 (subclass 2), SIRT5 (subclass 3) and finally SIRT6 and SIRT7 (subclass 4) (Fig. 5b, Table II) (1-3).

Sirtuin 1 (SIRT1), which is a member of the Sir2 family of NAD^+-dependent histone deacetylases, deacetylates lysines 9 and 14 of histone H3 and specifically lysine-16 of histone H4, while it hydrolyzes one molecule of NAD^+ for every lysine residue that is deacetylated (4). Derivatives of the yeast Sir2 histone deacetylase share a common catalytic domain, which is highly conserved in organisms ranging from bacteria to humans and which is composed of two distinct motifs that bind NAD^+ and the acetyl-lysine substrate, respectively (5). SIRT1, like Sir2, has been shown to directly modify chromatin and silence transcription (6-10), to modulate the meiotic checkpoint (11), and as a probable antiaging effect, to increase genomic stability and to suppress rDNA recombination (8,12,13). While for yeast Sir2 no targets are known apart from histones, SIRT1 has a large and still growing list of targets, which includes p53 and forkhead transcription factors, which are mammalian homologs of Daf-16 and which are known to function as sensors of the insulin signaling pathway (8,14).

In yeast, Sir2 has been reported to directly link the cellular energy status to longevity (8,15). Calorie restriction induces a metabolic switch that increases the NAD/NADH ratio and/or decreases levels of nicotinamide, which is a Sir2 inhibitor and therefore activates Sir2 and increases rDNA stability (8,16). Similarly, during calorie restriction in mammals SIRT1 activates fat mobilization in white adipocytes and represses genes that mediate fat storage and genes being controlled by the fat regulator PPAR-γ by docking with its cofactors NCOR and SMRT (17). An overexpression or pharmacologic activation of yeast Sir2 related proteins has been reported to extend the life-span in a number of different organisms (18-20), which depends on the presence of the transcription factor Daf-16 (19), an important mediator in the insulin-like signaling pathway, which is usually employed by worms to hold out as extremely long-lived larvae under unfavorable environmental conditions (21,22). The SIRT1 protein binds, deacetylates and reduces the transcriptional

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Abbreviations: HDAC, histone deacetylase; HAT, histone acetyltransferase

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activity of p53 and p53-mediated functions (23,24), an effect, which is inhibited by nicotinamide (vitamin B3). Furthermore, SIRT1 is known to deacetylate and repress the activity of the mammalian forkhead transcription factor FOXO3A and to reduce both, forkhead-dependent and p53-dependent apoptosis in response to DNA damage and oxidative stress (25). Acute nutrient withdrawal in mammalian cells has been reported to activate FOXO3A and to increase FOXO3A-mediated expression of SIRT1, which is dependent on the presence of two p53 binding sites in the SIRT1 promoter, and a nutrient-sensitive physical interaction that was observed between FOXO3A and p53. More recently, SIRT1 has been reported to exhibit a dual effect on FOXO3 function: while on one hand SIRT1 increases the FOXO3 ability to induce cell cycle arrest and resistance to oxidative stress, it does on the other hand inhibit the FOXO3-mediated induction of cell death, which consequently results in an increase of organismal longevity (14,26,27).

Three classes of small molecules, of which resveratrol, a polyphenol that is found in red wine, is the most potent, have been found to mimic calorie restriction and to activate sirtuins by lowering the Michaelis constant of SIRT1 for both the acetylated substrate and NAD^+ and to subsequently extend the cellular life-span through stimulation of SIRT1-dependent deacetylation of p53 (28,29).

Materials and methods

Identification of the human Sirt1 cDNA. A homology search of the EST database at NCBI (National Center for Biotechnology Information) yielded eight positive cDNA clones: GenBank AF083106 (4,086 bp); AF235040 (4,040 bp); AK027686 (3,142 bp), AK074805 (3,284 bp), AL136741 (3,719 bp), BC012499 (3,691 bp) BX648554 (3,345 bp), and NM_012238 (4,107 bp), of which GenBank clone NM_012238 was obtained from the Reference Center of the German Human Genome Project (RZPD, Berlin, Germany). The authenticity of its insert was confirmed by DNA cycle sequencing.

Identification of BAC genomic clone RZPDB737C042021D. The human *Sirt1* genomic clone was obtained from an arrayed BAC genomic library (Human Genomic Set - RZPD 1.0) after *in silico* screening with the *Sirt1* cDNA (GenBank clone NM_012238), which was shown to contain the full-length human *Sirt1* cDNA. BAC clone *RZPDB737C042021D* was identified to contain inserts with an average size of ~120 kb in the vector pBACe3.6, which included the human *Sirt1* genomic sequence. BAC genomic DNA was prepared according to published protocols (30) and the *Sirt1* insert was confirmed by cycle sequencing (31).

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 (31). 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). Standard chromosome preparations were used from a human lyphoblastoid cell line. 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 where then washed 2x10 min in 1X PBS and 1x10 min in 1X PBS/50 mM $MgCl_2$ at room temperature. 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 about 300 bp after nick translation. Approximately 50 ng DNA were pooled 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 at 72˚C for 3 min. Hybridization was over night 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 72˚C in 0.4X SSC/0.1% Tween-20 for 1 min. The slide was then washed shortly in 2X SSC at room temperature and stained in DAPI (4',6-diamidino-2-phenylindole) for 10 min. For microscopy the slide was mounted in antifade solution (Vectashield). *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 (DKFZ, Heidelberg) 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 (32). 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 (Fig. 5, Table II) (33). 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

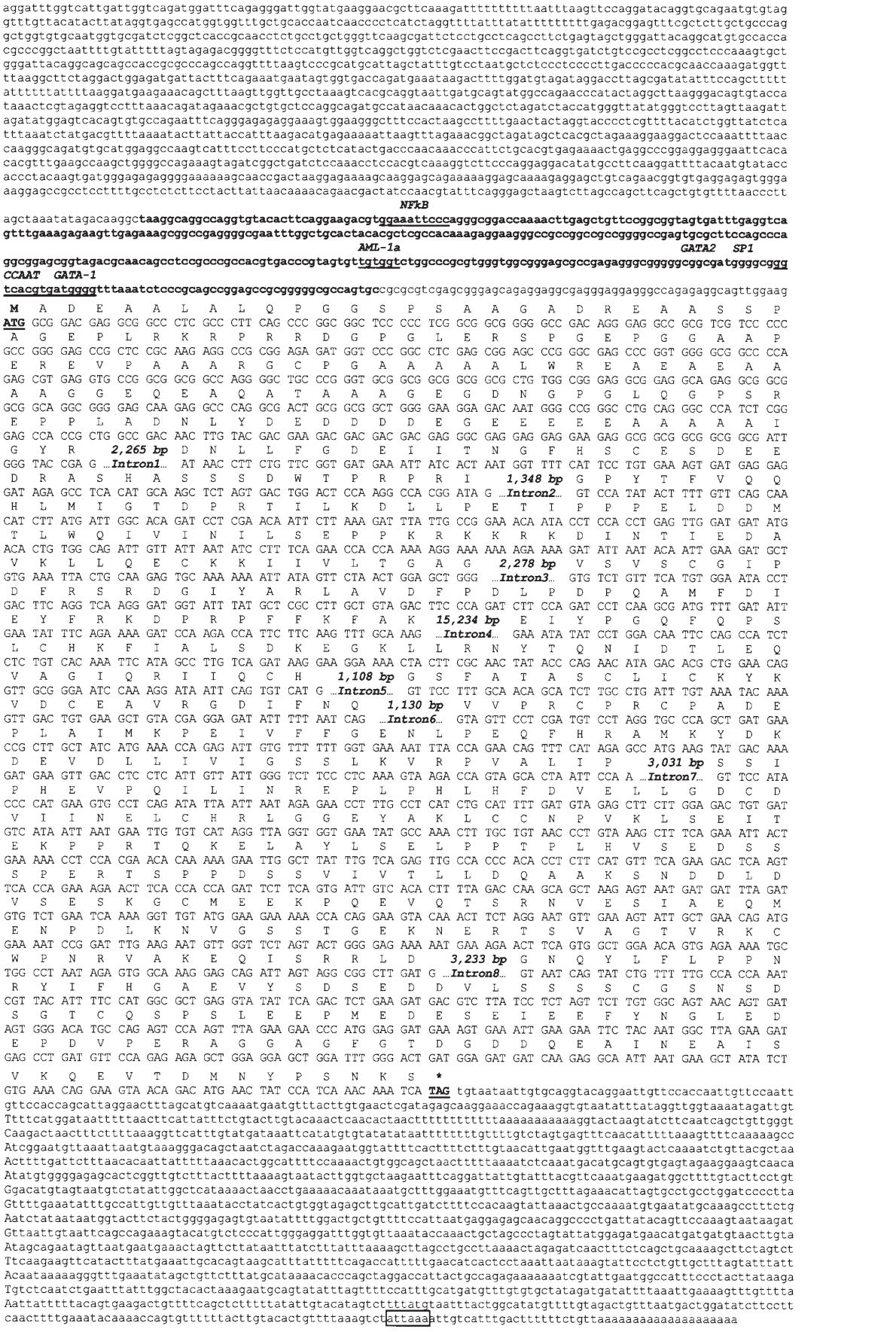

Figure 1. The complete sequence of *Sirt1* cDNA together with the predicted amino acid sequence is shown with the location of each intron with respect to the cDNA sequence. The 4,107 bp human *Sirt1* mRNA has an open reading frame of 2,244, which yields a 747 aa protein and an untranslated 3' flanking region, which is 1,782 bp long (distance from the translational termination codon to the polyadenylation signal). 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. A small CpG island that measures approximately 350 bp in length, is shown in fat lower case. The translational start (ATG) and stop codons (TGA) are underlined, the polyadenylation signal (ATTAAA) is boxed.

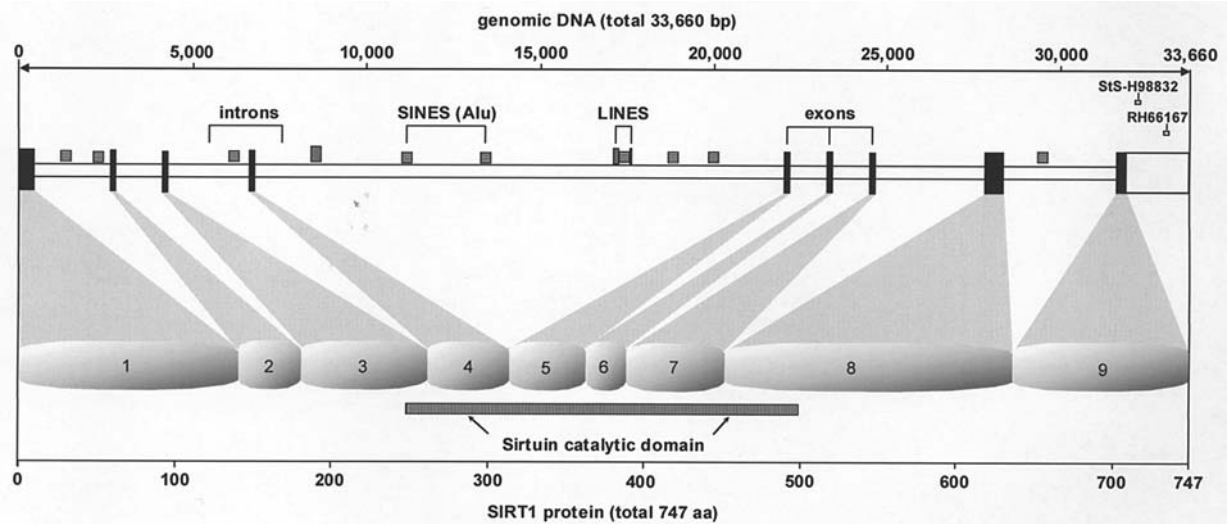

Figure 2. Genomic organization of the human *Sirt1* gene. The genomic organization of the 33.7 kb long *Sirt1* gene, which includes the relative position of exons and introns is shown. Repetitive sequences, known as short and long interspersed nuclear elements (SINEs and LINEs) are indicated. The 3' untranslated flanking region of exon 9 contains STS-marker STS-H98832 and RH66167. 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 human *Sirt1* gene.

Exon no.	Exon size	5'-splice donor	Intron no.	Intron size	3'-splice acceptor
1	430	GGGTACCGAG**gt**gcgcagggtg	1	2,265	tttcttttgc**ag**ATAACCTTCT
2	117	CCACGGATAG**gt**atggctcaga	2	1,348	cccttattgt**ag**GTCCATATAC
3	242	TGGAGCTGGG**gt**atgtaagact	3	2,278	tgcacattttt**ag**GTGTCTGTTT
4	153	GTTTGCAAAG**gt**actatgaact	4	15,234	tgcttgatac**ag**GAAATATATC
5	148	CAGTGTCATG**gt**tagtaaactt	5	1,108	gtttgttttt**ag**GTTCCTTTGC
6	80	TTTTAATCAG**gt**aatttgttgc	6	1,130	gaaaatatgt**ag**GTAGTTCCTC
7	186	CTAATTCCAA**gt**aagttggtga	7	3,031	accctatttt**ag**GTTCCATACC
8	558	CGGCTTGATG**gt**aagaaaggca	8	3,233	actgtatttc**ag**GTAATCAGTA
9	2,120				

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.

GenomeNet WWW server at Institute for Chemical Research, Kyoto University (Japan), but still remain to be experimentally 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 (34). 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 known human class I through class IV histone deacetylase sequences, which were obtained from a protein sequence similarity search with the human SIRT1 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 (35). Trees were calculated and drawn with the PATH (phylogenetic analysis task in HUSAR) software, which estimates and realizes phylogenies by executing the three main phylogenetic methods: distance, parsimony and maximum likelihood and which is hosted by the HUSAR (Heidelberg Unix Sequence Analysis Resources) server from the Biocomputing Service Group at the German Cancer Research Center (DKFZ, Heidelberg) (Fig. 5a). For Fig. 5b trees were calculated and drawn with the PileUp software, which computes a multiple sequence alignment using a simplification of the progressive alignment method of Feng and Doolittle (36) and which can plot a dendrogram like the one illustrated, that shows the clustering relationships used to determine the order of the pairwise alignments that together create the final

sequence alignment. Distance along the vertical axis ional to the difference between sequences; distance along the horizontal axis has no significance at all (3).

Results

Identification and cloning of cDNAs encoding human Sirt1. Homology searches of the dbEST at NCBI (National Center for Biotechnology Information) (32) for the *Sirt1* cDNA sequence (1) yielded eight positive cDNA clones: GenBank AF083106 (4,086 bp); AF235040 (4,040 bp); AK027686 (3,142 bp), AK074805 (3,284 bp), AL136741 (3,719 bp), BC012499 (3,691 bp) BX648554 (3,345 bp), and NM_012238 (4,107 bp), of which GenBank clone NM_012238 was obtained from the Reference Center of the German Human Genome Project (RZPD, Berlin, Germany). 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* human genomic clone BAC *RZPDB737C042021D*. Characterization of the 5' flanking genomic region, which precedes the *Sirt1* open reading frame, revealed a CCAAT-box and a number of putative optimal transcription factor binding sites for NF-κB and GATA in addition to a small 350 bp CpG island, their biological relevance awaits still to be investigated experimentally. The 4,107 bp human *Sirt1* mRNA encodes a 747 aa protein with a predictive molecular weight of 81.7 kDa and an isoelectric point of 4.55. Fluorescence *in situ* hybridization analysis localized the human *Sirt1* gene to chromosome 10q21.3. Translational stop codons in all reading frames precede the human *Sirt1* open reading frame. The 3' flanking region was shown to contain the eukaryotic polyadenylation consensus signal ATTAAA 1,782 bp downstream of the termination of translation signal TAG (Fig. 1) (37).

Identification and characterization of the human Sirt1 genomic locus. The human *Sirt1* genomic clone was obtained from an arrayed BAC genomic library (Human Genomic Set - RZPD 1.0) after *in silico* screening with the *Sirt1* cDNA (GenBank clone NM_012238), which was shown to contain the full-length human *Sirt1* cDNA. BAC clone *RZPDB737C042021D* was identified to contain inserts with an average size of ~120 kb in the 11.6 kb vector pBACe3.6, which included the human *Sirt1* genomic sequence. BAC genomic DNA was prepared according to published protocols (30) and the *Sirt1* insert was confirmed by cycle sequencing (31). Genomic sequence comparison analyses with the BLAST algorithm helped us with the identification of human chromosome 10 genomic contig NT_025826, which is part of the third release of the finished human reference genome and which was assembled from individual clone sequences by the Human Genome Sequencing Consortium together with NCBI. We have used this sequence for the determination of *Sirt1* introns and exon/intron boundaries (Table I). The human *Sirt1* gene spans a region of 33,660 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 2,120 bp (exon 9). Within introns 1, 3, 8 and 4 in particular, we identified an accumulation of interspersed repetitive elements, SINEs (short interspersed nuclear elements) and LINEs (long interspersed nuclear elements) (Fig. 2). Additionally, we have

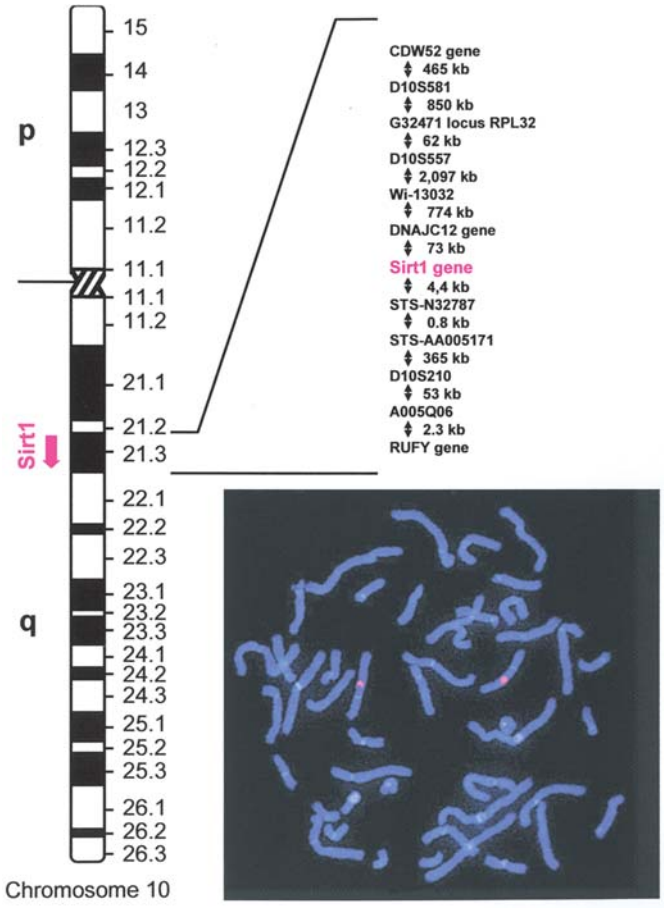

Figure 3. Chromosomal mapping of the human *Sirt1* gene. Lower right panel, fluorescence *in situ* hybridization of BAC clone *RZPDB737C042021D* to human chromosome 10q21.3. Left panel, chromosome 10 idiogram according to the International System for Cytogenetic Nomenclature (ISCN 1995), which illustrates the chromosomal position of BAC clone *RZPDB737C042021D* within the interval D10S581 to D10S210. Human *Sirt1* is closely neighbored by the DNAJC12 gene, which is located 73 kb towards the chromosome 10 centromere and the RUFY gene, which is located ~426 kb towards the chromosome 10 telomere. Neighboring markers are also indicated. The chromosomal orientation of *Sirt1* is shown (red arrow).

identified an internal STS-marker *RH66167* alias *STS-H98832* within the untranslated proportion of exon 9 of *Sirt1* 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 244 and 498, i.e. within exons 3 and 8 of the protein (Fig. 2).

Sirt1 is a single copy gene. Both sequencing and results obtained by electronic PCR of BAC clone *RZPDB737C042021D* identified STS markers StS-H98832 and RH66167 within exon 9 at the very 3' end of the *Sirt1* genomic sequence. Our fluorescence *in situ* hybridization studies localized *Sirt1* to chromosome 10q21.3. These data, together with the results obtained by electronic PCR and the already known location of STS markers StS-H98832 and RH66167, indicated one single site of hybridization of *Sirt1* on human metaphase chromosomes and its specific localization on chromosome 10q21.3 (Fig. 3).

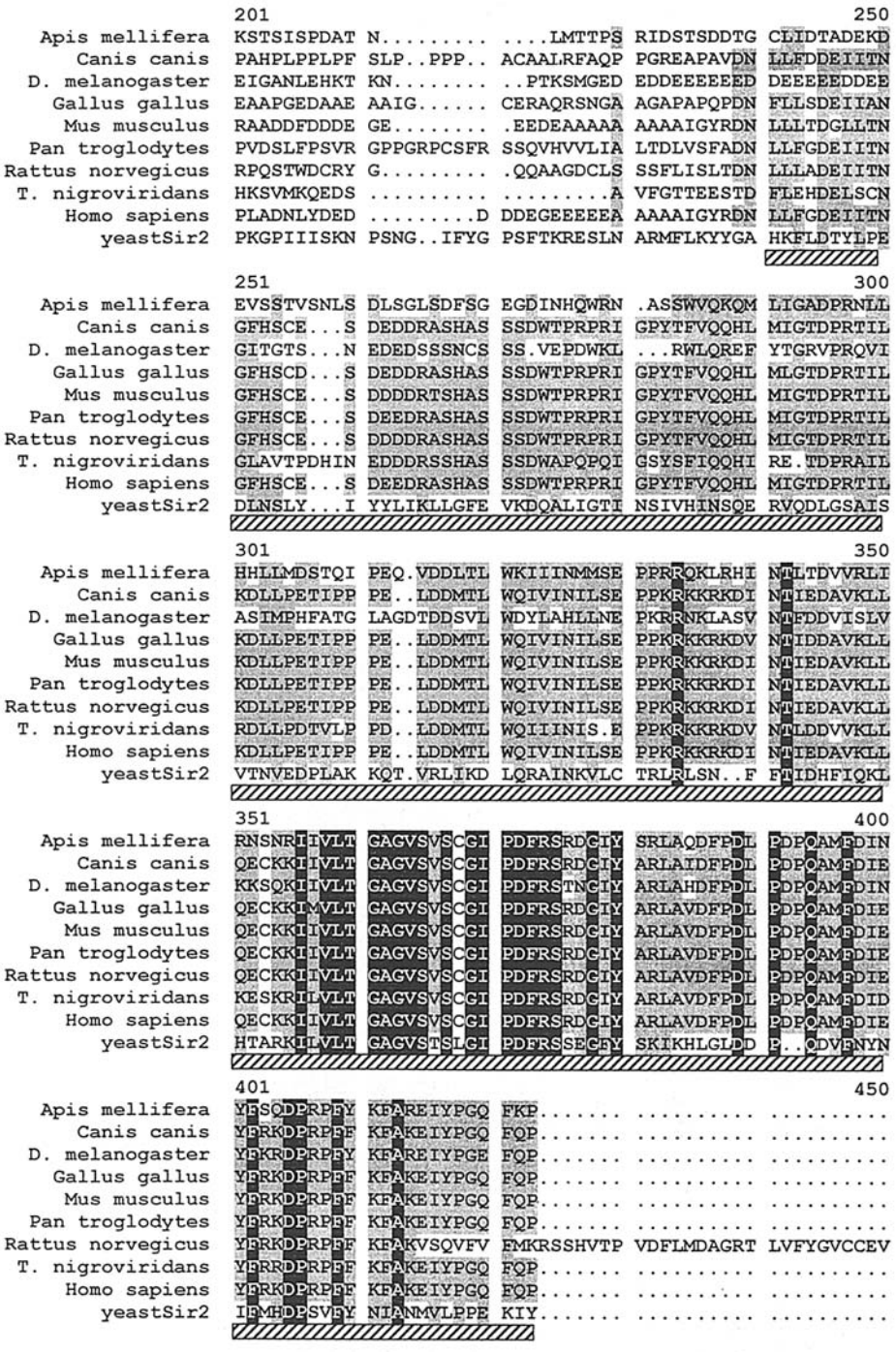

Figure 4. Amino acid sequence alignment of human SIRT1 catalytic domain from different species. CLUSTAL W colors mark similarities in protein sequences. Black background, >75% of nucleotides of a column are identical; grey, more than half of the amino acids of a column are identical or belong to one of the strong groups (amino acids with strong similarities). The sirtuin catalytic domain is indicated by a horizontal box. Accession numbers of the sequences used in this tree: 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), yeast Sir2 (GenPept P06700).

Sirt1 expression analyses. *In silico* expression profile analyses have been carried out with the UniGene EST profile viewer, which is hosted by the NCBI homepage and which suggested the strongest expression of *Sirt1* in the thymus, followed by vascular cells, lymph nodes and small intestine cells on the basis of an analysis of EST counts. Additional analyses were carried out with help of the Human GeneAtlas Gene Expression Database, which is being hosted by the Genomics Institute of the Novartis Research Foundation ('GNF') and which showed the strongest accumulation of human SIRT1 in whole blood and more specifically in healthy lymphocytes (T-cells, B-cell and NK-cells), in lymphoma cells and in myeloid bone marrow cells (38-40).

Phylogenetic analysis and pairwise sequence comparisons. We have screened the expressed sequence tag database (NCBI)

Sequence identity and similarity among class III sirtuin-HDACs (the indicated numbers represent the percentage of identity and similarity from pairwise sequence comparisons).

	Human SIRT1	Human SIRT2	Human SIRT3	Human SIRT4	Human SIRT5	Human SIRT6	Human SIRT7	Yeast SIR2
Human SIRT1		42	40	30	28	22	23	40
Human SIRT2	65		50	26	27	27	25	31
Human SIRT3	63	66		28	31	28	28	35
Human SIRT4	47	43	43		27	28	28	25
Human SIRT5	43	44	43	46		21	24	26
Human SIRT6	39	44	40	43	36		42	23
Human SIRT7	39	42	40	45	37	56		21
Yeast SIR2	56	47	49	44	41	38	38	

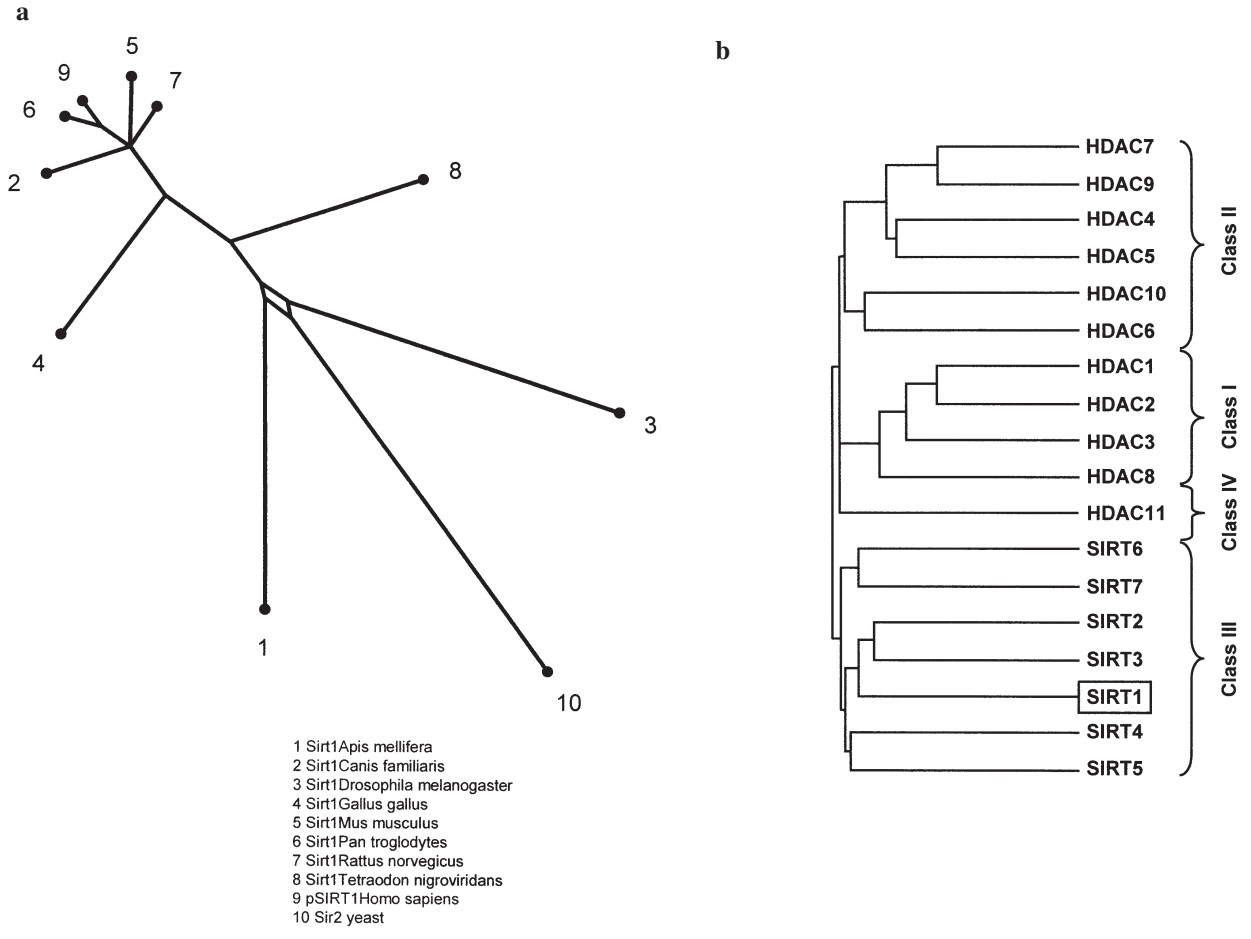

Figure 5. SIRT1 phylogenetic tree (a). This dendrogram depicts the sequence relatedness of the human SIRT1 protein with the SIRT1 proteins from different species. The GenPept accession numbers correspond to the ones that have also been used for the multiple sequence alignment as shown in Fig. 4. In (b) the position of human SIRT1 among the human orthologs for yeast RPD3, HDA1, SIR2 and HDAC11 related protein families of histone deacetylases is shown [accession nos. of the sequences used in this tree: human HDAC1 (GenPept Q13547), human HDAC2 (GenPept Q92769), human HDAC3 (GenPept O15379), human HDAC8 (GenPept AAF73428), human HDAC4 (GenPept AAD29046), human HDAC5 (GenPept AAD29047), human HDAC6 (GenPept AAD29048), human HDAC7 (GenPept AAF04254), human HDAC9 (GenPept AAK66821), human HDAC10 (GenPept AAL30513), human HDAC11 (GenPept NP_079103), human SIRT1 (GenPept AAD40849), human SIRT2 (GenPept AAD40850), human SIRT3 (GenPept AAD40851), human SIRT4 (GenPept AAD40852), human SIRT5 (GenPept AAD40853), human SIRT6 (GenPept AAF43432) and human SIRT7 (GenPept AAF43431)].

with the human SIRT1 protein sequence and identified several yeast and human histone deacetylases, which share a significant degree of sequence identity with SIRT1, indicating a high degree of phylogenetic conservation of protein structure and associated function throughout evolution. A consensus evolutionary tree was obtained using an alignment of yeast SIR2 with a selection of different mammalian and non-mammalian SIRT1 homologs (Fig. 5a). In addition, a dendgrogram has been calculated from class I through class IV human sirtuin and non-sirtuin HDACs (Fig. 5b) (3). Obviously, the sirtuin family of HDACs (class III) does not reveal significant sequence homology with the three classes of non-sirtuin HDACs. The tree was constructed after bootstrapping and clearly identifies four families of human histone deacetylases with HDAC1, HDAC2, HDAC3 and HDAC8 being members of the yeast RPD3 family of histone deacetylases (so-called 'mammalian class I histone deacetylases'), HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and HDAC10 being members of the yeast HDA1 family of histone deacetylases ('mammalian class II histone deacetylases') and SIRT1 through SIRT7 being homologs of the yeast SIR2 protein ('mammalian class III histone deacetylases'), while HDAC11 is so far the only member of a distinct group of class IV HDACs (Fig. 5b) (3).

Discussion

A dysregulation of the tightly controlled equilibrium of acetylation and deacetylation plays a causative role in the generation as well as in the suppression of cancer (41). Histone acetylation modifiers are therefore gaining increasing attention as potential targets in the treatment of cancer. Relaxation of the chromatin fiber facilitates transcription and is regulated 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, lead to 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 known to be involved both in the pathogenesis as well as 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 (41). On the other hand, some histone acetylation modifying enzymes have been located within chromosomal regions that are particularly prone to chromosomal breaks. In these 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 (42).

SIRT1 is a human ortholog to yeast Sir2 and is an NAD^+-dependent deacetylase (4). The protein is localized in the nucleus (23,24), and interacts with and deacetylates a growing number of proteins, such as p53 (23,24), the forkhead transcription factor FOXO3A (25), PML (43), BCL6 (44), TAF_I68 (45), HES1 (46), and CTIP2 (47). SIRT1 has been shown to be essential for embryonic development (21,22), muscle differentiation (48) 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. It is now important to determine whether SIRT1, in addition to silencing transcription, also suppresses recombination and genomic instability via chromatin effects and if so, whether such an activity could be involved in regulating aging and whether it potentially mediates an anti-tumor effect in mammals.

In the present study, we report the cloning, characterization and mapping of *Sirt1* on the genomic level. Human *Sirt1* is a single-copy gene that spans a region of approximately 33.7 kb. It is composed of 9 exons (Table I) ranging in size from 80 bp (exon 6) to 2,120 bp (exon 9) and reveals an accumulation of interspersed repetitive elements within introns 1, 3, 8 and 4 in particular, 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) (49). The histone deacetylase catalytic domain is highly conserved within all members of mammalian HDACs that have been described so far and is located within exons 3 through 8 (Fig. 2). The 5' upstream *Sirt1* promoter region was found to contain a CCAAT-box and a number of NF-κB and GATA transcription factor binding sites in addition to a small 350 bp CpG island (Fig. 1). The human *Sirt1* mRNA encodes a 747 aa protein with a predictive molecular weight of 81.7 kDa. The pairwise comparison of sirtuin and non-sirtuin HDAC sequences clearly shows that sirtuin-HDACs are a distinct class of HDACs which does not share significant homology with the non-sirtuin HDACs (Fig. 5b) (3). Fluorescence *in situ* hybridization analysis in conjunction with electronic PCR localized the human *Sirt1* gene to the subband of chromosome 10q21.3 (Fig. 3). In view of the fact that the steady-state of histone acetylation and deacetylation plays a key role in the regulation of transcription, deletion of *Sirt1* would most probably shift the steady state toward acetylation at the level of specific genes targeted by SIRT1 and either upregulate or downregulate transcriptional events (41,50). Such dysregulation might represent a critical event in the multistep pathway leading to full cellular transformation and the development of malignancy.

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