Chromosomal organization and fluorescence in situ hybridization of the human Sirtuin 6 gene

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Received August 17, 2005; Accepted October 21, 2005

Abstract. Sirtuin 6 (SIRT6) is a member of the sirtuin deacetylases (sirtuins), which are derivatives of the yeast Silent information regulator 2 (Sir2) protein. SIR2 and its mammalian derivatives play a central role in epigenetic gene silencing, recombination, metabolism, cell differentiation and in the regulation of aging. In contrast to most sirtuins, SIRT6 lacks NAD+-dependent protein deacetylase activity. We have isolated and characterized the human Sirt6 genomic sequence, which spans a region of 8,427 bp and which has one single genomic locus. Determination of the exon-intron splice junctions found the full-length SIRT6 protein to consist of 8 exons ranging in size from 60 bp (exon 4) to 838 bp (exon 8). The human Sirt6 open reading frame encodes a 355-aa protein with a predictive molecular weight of 39.1 kDa and an isoelectric point of 9.12. Characterization of the 5’ flanking genomic region, which precedes the Sirt6 open reading frame, revealed a TATA- and CCAAT-box less promoter with an approximately 300-bp long CpG island. A number of AML-1 and GATA-x transcription factor binding sites were found which remain to be further evaluated experimentally. Fluorescence in situ hybridization analysis localized the human Sirt6 gene to chromosome 19p13.3; a region which is frequently affected by chromosomal alterations in acute leukemia. Human SIRT6 appears to be most predominantly expressed in bone cells and in the ovaries while, in the bone marrow, it is practically absent. The functional characteristics of SIRT6 are essentially unknown at present and remain to be elucidated.

Introduction

Based on structural and functional similarities, mammalian histone deacetylases are grouped into four categories, of which three contain non-sirtuin HDACs comprising the yeast histone deacetylases, RPD3 (class I HDACs), HDA1 (class II HDACs) and the more recently described HDAC11-related enzymes (class IV HDACs), while one category consists of sirtuin histone deacetylases (class III HDACs), which are homologs to the yeast Sir2 protein. SIRT1 is the mammalian sirtuin that is most closely related to S. cerevisiae SIR2. By contrast, mammalian SIRT6 is strongly related to SIRT7 (1) but only distantly homologous to human SIRT1 and yeast SIR2. The currently known seven human sirtuins have been further subgrouped into four distinct phylogenetic classes: SIRT1, SIRT2, and SIRT3 (subclass 1); SIRT4 (subclass 2); SIRT5 (subclass 3); and, finally, SIRT6 and SIRT7 (subclass 4, Fig. 1 and Table I) (1,2). Derivatives of the yeast SIRT2 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 (3,4). The yeast silent information regulator 2 protein (SIR2) is a NAD+-dependent histone deacetylase, which hydrolyzes one molecule of NAD+ for every lysine residue that is deacetylated (5). The yeast Sir2 protein, as well as its mammalian derivatives, has been shown to directly modify chromatin and to silence transcription (6-10), to modulate the meiotic checkpoint (11) and, as a probable anti-aging effect, to increase genomic stability and suppress DNA recombination (8,12,13). While yeast Sir2 exclusively targets histone proteins, mammalian SIRT1 has a large and growing list of targets, such as p53 and forkhead transcription factors, which are mammalian homologs of the Daf-16 protein, a key regulator within the insulin signaling pathway (8,14).

SIRT6 is a broadly expressed protein, which is predominantly found in the cell nucleus. In a tissue distribution analysis in the mouse embryo, SIRT6 reached peak levels at day E11, which further persisted into adulthood in muscle, brain and heart cells (15). The yeast silent information regulator 2 protein (SIR2); its mammalian orthologs, SIRT1, SIRT2, SIRT3, and SIRT5; and the bacterial protein, CobB; catalyze the tightly coupled cleavage of NAD+ and protein deacetylation, producing nicotinamide and 2-O-acetyl-ADP-
The deacetylase activity of sirtuin proteins is tightly coupled with their phosphoribosyltransferase activity and requires the presence of highly specific conserved amino-acid residues within the catalytic core of the protein, which are not contained in mammalian SIRT4, SIRT6, or SIRT7 and which are, therefore, lacking enzymatic deacetylase activity (2,8,15,17-19).

Calorie restriction is known to induce a metabolic switch that increases the NAD/NADH ratio and/or decreases levels of nicotinamide, which is a yeast SIR2 inhibitor, and as a result activates SIR2 and increases rDNA stability (8,20). Sirtuins, therefore, create a direct link between cellular energy status and longevity (8,21). Mammalian SIRT1 binds, deacetylates and reduces the activity of several transcription factors in vivo, including MyoD, p53, and FOXO, thereby affecting cell differentiation and survival under stress (14,22,23). The effect of SIRT1 on p53 may be inhibited by Nicotinamide (vitamin B3) (23-25). Calorie restriction in mammalian cells activates FOXO3A and increases FOXO3A-mediated expression of SIRT1, which depends 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 (14,26,27).

A cell that is low in energy will consume most of its NADH to generate ATP. The consequential high levels of nicotinamide adenine dinucleotide (NAD+) provide the indispensable cosubstrate for SIR2, which then activates acetyl-CoA synthetase (ACS) and subsequently results in the generation of more acetyl CoA, thus shunting more carbon into the NADH- and energy-generating TCA cycle (28). In addition to the generation of more acetyl CoA, active Sir2 is also known to extend lifespan. More acetyl CoA for the citric acid cycle means more respiration, which has been associated with yeast lifespan extension when caloric intake is restricted. Accordingly, when mammals are short of food they also alter their metabolism such that both aging and reproduction are postponed until better times (28).

In contrast to SIRT1, only minimal information is currently available on human SIRT6, which is a distantly related ortholog of yeast SIR2 (1,29), and which has been predicted to be predominantly a nuclear protein (78.3% nuclear, 13.0% cyto-

![Diagram of human SIRT6 among the human orthologs for yeast RPD3, HDAC1, SIR2 and HDAC11 related protein families of histone deacetylases (HDAC classes I-IV) and within the sirtuin-subclasses (subclasses I-IV) is shown (accession numbers of the sequences used in this tree: human HDAC1 (GenPept Q13547), human HDAC2 (GenPept Q92769), human HDAC3 (GenPept O15379), human HDAC8 (GenPept AA073428), human HDAC4 (GenPept AAD29046), human HDAC5 (GenPept AAD29047), human HDAC6 (GenPept AAD29048), human HDAC7 (GenPept AAF04254), human HDAC9 (GenPept AA66821), human HDAC10 (GenPept AAL30513), human HDAC11 (GenPept NP_079103), human SIRT1 (GenPept AAD40849), human SIRT2 (GenPept NP_036369), human SIRT3 (GenPept AAD40851), human SIRT4 (GenPept AAD40852), human SIRT5 (GenPept AAD40853), human SIRT6 (GenPept NP_057623) and human SIRT7 (GenPept AAF43431) (4).]

**Table I. Sequence identity and similarity among human class III sirtuin proteins.**

<table>
<thead>
<tr>
<th>Human SIRT1</th>
<th>Human SIRT2</th>
<th>Human SIRT3</th>
<th>Human SIRT4</th>
<th>Human SIRT5</th>
<th>Human SIRT6</th>
<th>Human SIRT7</th>
<th>Yeast SIR2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human SIRT1</td>
<td>42</td>
<td>40</td>
<td>30</td>
<td>28</td>
<td>22</td>
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<td>40</td>
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<td>Human SIRT2</td>
<td>65</td>
<td>50</td>
<td>26</td>
<td>27</td>
<td>27</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
<td>Human SIRT3</td>
<td>63</td>
<td>66</td>
<td>28</td>
<td>31</td>
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<td>Human SIRT5</td>
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<td>42</td>
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<td>Human SIRT6</td>
<td>39</td>
<td>44</td>
<td>40</td>
<td>43</td>
<td>36</td>
<td>42</td>
<td>23</td>
</tr>
<tr>
<td>Human SIRT7</td>
<td>39</td>
<td>42</td>
<td>40</td>
<td>45</td>
<td>37</td>
<td>56</td>
<td>21</td>
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<tr>
<td>Yeast SIR2</td>
<td>56</td>
<td>47</td>
<td>49</td>
<td>44</td>
<td>41</td>
<td>38</td>
<td>38</td>
</tr>
</tbody>
</table>

*The indicated numbers represent the percentage of sequence identity and similarity from pairwise sequence comparisons.*
skeletal, 4.3% Golgi, 4.3% mitochondrial) (30). Human SIRT6 appears to be most predominantly expressed in bone cells and in the ovaries, while in the bone marrow it is practically absent. The further functional characterization of mammalian SIRT6 may help to further elucidate its potential role in the mediation of stress resistance, anti-apoptosis, anti-aging and...
as a modulator of endocrine changes. In the study presented herein, we report the chromosomal localization and genomic organization of the human Sirt6 gene.

Materials and methods

Identification of the human Sirt6 cDNA. A homology search of the EST database at NCBI (National Center for Biotechnology Information) with the human Sirt6 cDNA that has been published earlier (1) yielded 7 positive cDNA clones of which one was obtained from the Reference Center of the German Human Genome Project (RZPD, Berlin, Germany). The authenticity of their inserts was confirmed by DNA cycle sequencing (Fig. 2).

Identification of BAC genomic clone, RZPDB737G031026D6. The human Sirt6 genomic clone was obtained from an arrayed BAC genomic library (Human Genomic Set - RZPD 1.0) after in silico screening with Sirt6 cDNA (GenBank clone NM_016539), which was shown to contain full-length human Sirt6 cDNA. BAC clone, RZPDB737G031026D6, was identified to contain inserts with an average size of approximately 120 kb in the vector, pBACE3.6, which included the human Sirt6 genomic sequence. BAC genomic DNA was prepared according to published protocols (31) and the Sirt6 insert was confirmed by cycle sequencing (32).

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 using an ABI PRISM 310 Genetic Analyzer which utilizes the four-color sequencing chemistry.

PCR methods. The human Sirt6 sequence was partially sequenced by primer walking on both strands using a direct sequencing strategy (32). Sequencing reactions were performed using 0.6 μg cDNA and 20-30mer 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), and DNA as indicated, and ddH₂O was added 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.

Sirt6 chromosomal localization by fluorescence in situ hybridization (FISH). Standard chromosome preparations were used from a human lymphoblastoid cell line. 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 for 2x10 min in 1X PBS and 1x10 min in 1X PBS/50 mM MgCl₂ at room temperature. BAC DNA was labeled by using 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 an optimal average length of ~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 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. The slide was then incubated at 72°C in 0.4X SSC/0.1% Tween for 1 min, 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 Axioscop 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 using SmartCapture X software (Digital Scientific, Cambridge, UK) (Fig. 4).

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 using the BLAST algorithm of the GenBank and EMBL databases (33). Protein similarity scores were calculated from fast alignments generated by the method of Wilbur and Lipman using the CLUSTAL W Multiple Alignment Program Version 1.7 (Figs. 1 and 5; Tables I and III) (34). 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 server at the Bioinformatics Center at the Institute for Chemical Research from the Kyoto University (Japan). Potential transcription factor binding sites were identified using the TRANSFAC program, which is part of the GenomeNet Computation Service (see above), but remain to be confirmed experimentally. Sequence similarities were calculated using 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 (35). Repetitive and CpG elements were identified on the RepeatMasker Server and with the CPG software hosted by the European Bioinformatics Institute (EMBL outstation) (Figs. 2 and 3).

Phylogenetic analysis. Phylogenetic trees were constructed from known human class I through class IV histone deacetylase sequences which were obtained from protein sequence similarity searches with the yeast proteins, RPD3, HDAl and SIR2, using the BLAST 2.0 program at NCBI database (Non-redundant GenBank CDS: translations+PDB+SwissProt+SPupdate+PIR). Progressive multiple sequence alignments were performed using the CLUSTAL W Multiple Alignment Program Version 1.7 (Fig. 1) (4,36). Trees were then calculated and drawn using PileUp software, which computes a multiple sequence alignment using a simplification of the progressive alignment method of Feng and Doolittle (37) and which can plot a dendrogram like the one below, that shows the clustering relationships used to determine the order of the pairwise alignments that together create the final multiple sequence
alignment. Distance along the vertical axis is proportional to the difference between sequences; distance along the horizontal axis has no significance at all. Trees in Fig. 5 were calculated and drawn using 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).

### Results

Identification and cloning of cDNAs encoding human Sirt6. Homology searches of the dbEST at NCBI (National Center for Biotechnology Information) (33) for the Sir6 cDNA sequence (1.2) yielded 7 positive cDNA clones: GenBank NM_016539 (1,638 bp), AF233396 (1,638 bp), AK074810 (1,603 bp), BC004218 (1,398 bp), BC005026 (1,600 bp), BC028220 (1,585 bp) and CR457200 (1,068 bp), of which GenBank clone NM_016539 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 (Fig. 2). Sequences flanking the 5' and 3' ends of the Sir6 open reading frame were identified from the Sir6 human genomic clone, BAC RZPDB737G031026D6. The human Sir6 mRNA is transcribed into a 1,638-bp mRNA with an open reading frame of 1,065 bp, which is translated into a 355-aa protein with a predictive molecular weight of 39.1 kDa and an isoelectric point of 9.12. Characterization of the 5' flanking genomic region, which precedes the Sir6 open reading frame, revealed a TATA- and CCAAT-box less promoter with an approximately 300-bp CpG island. A number of GATA-x and AML-1 transcription factor binding sites were found which remain to be further evaluated experimentally. Fluorescence in situ hybridization analysis localized the human Sir6 gene to chromosome 19p13.3. Translational stop codons in all reading frames precede the human Sir6 open reading frame. The 3' flanking region was shown to contain the eukaryotic polyadenylation consensus signal AATAAA (38), 484 bp downstream of the termination of translation signal TGA (Fig. 2).

Identification and characterization of the human Sir6 genomic locus. The human Sir6 genomic clone was obtained from an arrayed BAC genomic library (Human Genomic Set - RZPD 1.0) after in silico screening with Sir6 cDNA (GenBank clone NM_016539), which was shown to contain full-length human Sir6 cDNA. BAC clone RZPDB737G031026D6 was identified to contain inserts with an average size of approximately 120 kb in the 11.6-kb vector, pBACE3.6, which

![Figure 5. SIRT6 phylogenetic tree. This dendrogram depicts the sequence relatedness of the human SIRT6 protein with the SIRT6 homologs from different species. The GenPept accession numbers correspond to the ones that have also been used for the multiple sequence alignment as shown in Table III.](image)

### Table II. Exon/intron splice-junctions of the human Sir6 gene: exon sequences are given in uppercase and intron sequences are given in lowercase letters.

<table>
<thead>
<tr>
<th>Exon no.</th>
<th>Exon size</th>
<th>5'-splice donor</th>
<th>Intron no.</th>
<th>Intron size</th>
<th>3'-splice acceptor</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>66</td>
<td>CTTCCGGAAGtagcgcctctt</td>
<td>1</td>
<td>1.564</td>
<td>cttccccaccagATCTTGACC</td>
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<tr>
<td>2</td>
<td>128</td>
<td>CGACTTCCAGtgctgattgt</td>
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<td>1.491</td>
<td>tgcccaccctcagGGGTCCCCAC</td>
</tr>
<tr>
<td>3</td>
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<td>60</td>
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<td>81</td>
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</tr>
<tr>
<td>8</td>
<td>38</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aThe sizes of the single exons and introns are indicated. Consensus splice donor and splice acceptor sequences are given in bold.*
Table III. Amino-acid sequence alignment of the human SIRT6 catalytic domain with SIRT6 homologs from different species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Accession Number</th>
<th>Sequence Alignment</th>
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<tbody>
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<td>Anopheles gambiae</td>
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</tr>
<tr>
<td>Apis mellifera</td>
<td>GenPept XP_396298</td>
<td></td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td>GenPept XP_615499</td>
<td></td>
</tr>
<tr>
<td>Bos taurus</td>
<td>GenPept XP_542163</td>
<td></td>
</tr>
<tr>
<td>Canis familiaris</td>
<td>GenPept CAG30975</td>
<td></td>
</tr>
<tr>
<td>Dario rerio</td>
<td>GenPept AAH71405</td>
<td></td>
</tr>
<tr>
<td>Drosophila melanogaster</td>
<td>GenPept AAF54513</td>
<td></td>
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<td>Gallus gallus</td>
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<tr>
<td>Homo sapiens</td>
<td>GenPept NP_057623</td>
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</tr>
<tr>
<td>Mus musculus</td>
<td>GenPept NP_853617</td>
<td></td>
</tr>
<tr>
<td>Oryza sativa</td>
<td>GenPept XP_471492</td>
<td></td>
</tr>
<tr>
<td>Rattus norvegicus</td>
<td>GenPept AAF71405</td>
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</tr>
<tr>
<td>Tetraodon nigroviridis</td>
<td>GenPept CAG07749</td>
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<td>Zea mays</td>
<td>GenPept AAK67144</td>
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</tr>
</tbody>
</table>

ClustalW Colors mark similarities in protein sequences. Black background, more than 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). Accession numbers of the sequences used in this alignment: Anopheles gambiae (GenPept XP_321669), Apis mellifera (GenPept XP_396298), Arabidopsis thaliana (GenPept BAB09243), Bos taurus (GenPept XP_615499), Canis familiaris (GenPept XP_542163), Dario rerio (zebrafish, GenPept AAF71405), Drosophila melanogaster (GenPept AAF54513), Gallus gallus (GenPept CAG07749), Homo sapiens (GenPept NP_057623), Mus musculus (GenPept NP_853617), Oryza sativa (GenPept XP_471492), Rattus norvegicus (GenPept XP_234931), Tetraodon nigroviridis (GenPept CAG07749), Xenopus laevis (GenPept AAF71405), Zea mays (GenPept AAK67144), yeast Sir2 (GenPept P06700).
included the human Sirt6 genomic sequence. BAC genomic DNA was prepared according to published protocols (31) and the Sirt6 insert was confirmed by cycle sequencing (32). Genomic sequence comparison analyses with the BLAST algorithm helped us with the identification of human chromosome 6 genomic contig NT_011245, which was sequenced and assembled from individual clone sequences by the Human Genome Sequencing Consortium together with NCBI. We have used this sequence for the determination of Sirt6 introns and exon/intron boundaries (Table II). The human Sirt6 gene spans a region of 8,427 bp (Fig. 3). Determination of the exon-intron splice junctions found the full-length SIRT6 protein to consist of 8 exons ranging in size from 60 bp (exon 4) to 838 bp (exon 8). Within introns 1, 3 and 4 we identified an accumulation of interspersed repetitive elements, SINEs (short interspersed nuclear elements) (Fig. 3). Additionally, we have identified an internal STS-marker, sTSG48370, which is located within the untranslated proportion of exon 8. 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 52 and 221, i.e. within exons 2 and 7 of the protein (Fig. 3).

Sirt6 is a single copy gene. Sequencing and results obtained by electronic PCR of BAC clone RZPDB737G031026D6 identified STS-marker sTSG48370 to be located within the Sirt6 genomic sequence. These data, together with the results obtained by electronic PCR and the reported location of the mentioned STS-markers, indicated one single site of hybridization of Sirt6 on human metaphase chromosomes and its specific localization on chromosome 19p13.3 (Fig. 4).

Sirt6 expression analyses. In silico expression profile analyses were carried out using the UniGene EST profile viewer, which is hosted by the NCBI homepage, and the Human GeneAtlas Gene Expression Database, which is hosted by the Genomics Institute of the Novartis Research Foundation (GNF) and which identified human SIRT6 to be most predominantly expressed in bone cells and in the ovaries but practically absent in bone marrow (39–42).

Phylogenetic analysis and pairwise sequence comparisons. We have screened the 'all non-redundant GenBank CDS translations + RefSeq Proteins + PDB + SwissProt + PIR + PRF expressed sequence tag database' ('nr' at NCBI) with
the human SIRT6 protein sequence and identified several yeast and human histone deacetylases which were sharing a significant degree of sequence homology with human SIRT6, indicating a high degree of phylogenetic conservation of protein structure and associated function throughout evolution. The tree was constructed after bootstrapping and depicts a subdivision into four main evolutionary branches (plants, insects, fish and mammals) (Fig. 5). In an additional analysis, a consensus evolutionary tree was obtained for class I through class IV human sirtuin and non-sirtuin HDACs on the basis of an alignment of the yeast RPD3, HDA1, SIR2 and HDAC11 human homologous proteins (Fig. 1). 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. 1) (4).

**Discussion**

The human Sirt6 gene encodes members of the sirtuin family of proteins which are referred to as class III NAD+-dependent histone deacetylases on the basis of their homology to the yeast Sir2 protein (5). The members of the sirtuin family are characterized by a sirtuin core domain and are grouped into four subclasses with SIRT6 being a class IV sirtuin member (Fig. 1). For most of the currently known human sirtuins, a function has not yet been determined. In yeast, however, sirtuin proteins are known to regulate epigenetic gene silencing and suppress recombination of rDNA. In addition to their deacetylating activity, human sirtuins may function as intra- cellular regulatory proteins with mono-ADP-ribosyltransferase activity (2). Human SirT6 has been predicted to be predominantly a nuclear protein (30), which seems to be strongly expressed in bone cells and in the ovaries, while being practically absent in bone marrow (39-42). In the mouse embryo, Sirt6 has been reported to reach peak levels at day E11, which further persisted into adulthood in muscle, brain and heart cells (15).

In the present study, we report the identification, cloning and mapping of Sirt6 on the genomic level. Human Sirt6 is a single-copy gene that spans a region of approximately 8.5 kb. It is composed of 8 exons (Fig. 3 and Table II) ranging in size from 60 bp (exon 4) to 838 bp (exon 8) and reveals an accumulation of interspersed SINEs (Alu repeats) within introns 1, 3, and 4 (43). The SIR2 family domain is highly conserved within all members of mammalian sirtuin proteins that have been described so far and is located within exons 2 through 7 (Fig. 3). The 5’ upstream Sirt6 promoter region was found to contain a small 300 bp CpG island and lacks the canonical TATA-and CCAAT boxes (Fig. 2). TATA-independent transcription in the presence of accumulated CpG elements has been described to be a typical feature of constitutively active housekeeping genes (44). Human Sirt6 mRNA encodes a 355-aa protein with a predictive molecular weight of 39.1 kDa. Fluorescence *in situ* hybridization analysis in conjunction with electronic PCR localized the human Sirt6 gene to the sub-band of chromosome 19p13.3 (Fig. 4), a region which has been found to be involved in numerous chromosomal abnormalities in association with malignant disease, especially in acute leukemias (403 of 522 cases) (Fig. 6). These data have been retrieved from the Cancer Genome Anatomy Project (CGAP) database at the National Cancer Institute (45).

It is currently not clear to what extent chromosomal abnormalities that involve the chromosome 19p13 chromosomal region have an influence on SIRT6-mediated functional effects. It is, however, evident that a number of sirtuin proteins are located within chromosomal regions that are particularly prone to chromosomal breaks. In such cases, gains and losses of chromosomal material may affect the availability of functionally active sirtuin proteins, which in turn disturbs the tightly controlled intracellular equilibrium of protein acetylation and/or ADP ribosylation, respectively (46). Protein 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 results in chromatin condensation and the transcriptional repression of specific target genes.

Unfortunately, it is currently not possible to assess to what extent human SIRT6 is playing a role in the pathogenesis of hematological malignancies and acute myeloid leukemia in particular. It is, however, evident that SIRT6 contains multiple repetitive elements at the genomic level, which makes the region particularly prone to chromosomal breaks, while it is located within a chromosomal region that is known to be frequently part of chromosomal alterations in acute leukemia. In the context of such chromosomal modifications that may involve SIRT6, the SIRT6 protein could potentially be either missing, dysfunctional or exhibit its enzymatic activity at wrong times in the wrong places and therefore contribute to an imbalance of the intracellular acetylation status and to the development of disease. The further characterization of the functional role of human SIRT6 is therefore likely to become an exciting endeavor.

**Acknowledgements**

This work was supported by the German National Science Foundation (Deutsche Forschungsgemeinschaft, MA 2057/2-4).

**References**