

Minireview

Sirtuin/Sir2 Phylogeny, Evolutionary Considerations and Structural Conservation

Sebastian Greiss, and Anton Gartner*

The sirtuins are a protein family named after the first identified member, *S. cerevisiae* Sir2p. Sirtuins are protein deacetylases whose activity is dependent on NAD⁺ as a cosubstrate. They are structurally defined by two central domains that together form a highly conserved catalytic center, which catalyzes the transfer of an acetyl moiety from acetyllysine to NAD⁺, yielding nicotinamide, the unique metabolite O-acetyl-ADP-ribose and deacetylated lysine. One or more sirtuins are present in virtually all species from bacteria to mammals. Here we describe a phylogenetic analysis of sirtuins. Based on their phylogenetic relationship, sirtuins can be grouped into over a dozen classes and subclasses. Humans, like most vertebrates, have seven sirtuins: SIRT1–SIRT7. These function in diverse cellular pathways, regulating transcriptional repression, aging, metabolism, DNA damage responses and apoptosis. We show that these seven sirtuins arose early during animal evolution. Conserved residues cluster around the catalytic center of known sirtuin family members.

INTRODUCTION

The sirtuins are a family of NAD⁺-dependent deacetylases. They are named after their founding member budding yeast Sir2p, a histone deacetylase (Braunstein et al., 1993; 1996) first discovered in a genetic screen for genes required for transcriptional silencing of the budding yeast mating type loci (Ivy et al., 1985; 1986; Klar et al., 1979). In animals, sirtuins have also been implicated in transcriptional silencing (Astrom et al., 2003; Newman et al., 2002; Pruitt et al., 2006; Rosenberg and Parkhurst, 2002; Tissenbaum and Guarente, 2001; Vaquero et al., 2004; 2007), aging (Rogina and Helfand, 2004; Tissenbaum and Guarente, 2001; Wood et al., 2004) and metabolic regulation (Schwer and Verdin, 2008). The sirtuin family appears to be virtually ubiquitous throughout all kingdoms of life and the number of distinct sirtuins within an organism ranges from as little as one in bacteria to seven in vertebrates.

The biological functions of sirtuins have been summarized in several recent reviews (Dali-Youcef et al., 2007; Haigis and Guarente, 2006; Longo and Kennedy, 2006; North and Verdin, 2004; Schwer and Verdin, 2008; Yamamoto et al., 2007). The founding member, budding yeast Sir2p was initially shown to be

required for the transcriptional silencing of the mating type loci, and subsequently also implicated in transcriptional silencing at telomere proximal sites (Aparicio et al., 1991) and at ribosomal repeats (Bryk et al., 1997; Fritze et al., 1997; Gottlieb and Esposito, 1989). Sir2p forms complexes with different protein cofactors depending on the target site. At telomeres and the mating type loci, Sir2p forms a complex with Sir3p and Sir4p (Aparicio et al., 1991), while at rDNA sites Sir2p associates with Net1p and Cdc14p to form the regulator of nucleolar silencing and telophase exit (RENT) complex (Shou et al., 1999; Straight et al., 1999). In budding yeast, Sir2p action at ribosomal repeats is required to prevent illegitimate recombination leading to extrachromosomal ribosomal DNA circles, the accumulation of which is associated with aging (Gottlieb and Esposito, 1989; Kaerberlein et al., 1999; Sinclair and Guarente, 1997).

In animals, sirtuins have been implicated in a wide variety of processes, including transcriptional silencing (Astrom et al., 2003; Newman et al., 2002; Pruitt et al., 2006; Rosenberg and Parkhurst, 2002; Tissenbaum and Guarente, 2001; Vaquero et al., 2004; 2007), aging (Rogina and Helfand, 2004; Tissenbaum and Guarente, 2001; Wood et al., 2004), metabolic regulation (Schwer and Verdin, 2008), and apoptosis (Cohen et al., 2004; Dai et al., 2007; Greiss et al., 2008; Luo et al., 2001; Vaziri et al., 2001; Wang et al., 2006). Many of these functions are not related to histone deacetylation, and multiple non-histone substrates have been identified. Mammalian sirtuins (SIRT1–7) share the conserved sirtuin domain but vary in subcellular localization and function. SIRT1, SIRT6 and SIRT7 localize to the nucleus, SIRT3, SIRT4 and SIRT5 are mitochondrial, while SIRT2 is predominantly cytoplasmic (Michishita et al., 2005). SIRT1, the best characterized mammalian sirtuin, is predominantly nuclear but also has roles in the cytoplasm (Jin et al., 2007; Tanno et al., 2007). SIRT1 interacts with and regulates a number of histone and non-histone protein substrates including p53 (Luo et al., 2001; Vaziri et al., 2001), NF- κ B (Yeung et al., 2004), PPAR γ (Picard et al., 2004), PGC-1 α (Rodgers et al., 2005) and Foxo transcription factors (Brunet et al., 2004; Motta et al., 2004; van der Horst et al., 2004). It has roles in developmental and aging regulation (Yamamoto et al., 2007), and recent evidence also points towards a role in ensuring efficient DNA double-strand break repair (Oberdoerffer et al., 2008). SIRT6 has been found to deacetylate histone H3 and is linked to transcriptional regulation and the maintenance of ge-

Wellcome Trust Centre for Gene Regulation and Expression, University of Dundee, Dundee DD1 5EH, United Kingdom

*Correspondence: a.gartner@dundee.ac.uk

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nomic stability (Kawahara et al., 2009; Lombard et al., 2008; Michishita et al., 2008). SIRT7, which is present in the nucleus, regulates RNA-PolII mediated expression of ribosomal RNA genes and plays a role in angiogenesis (Ford et al., 2006; Potente et al., 2007). SIRT2 may have a role in cell cycle regulation, is able to deacetylate histone H4, and appears to act as a tumor suppressor in certain gliomas (Dryden et al., 2003; Hiratsuka et al., 2003; Inoue et al., 2006; Vaquero et al., 2006). Little is known about the mitochondrial sirtuins SIRT3, SIRT4 and SIRT5, although specific substrates that have been identified so far include acetyl coenzyme A synthetase 2, glutamate dehydrogenase and cytochrome c respectively (Haigis et al., 2006; Hallows et al., 2006; Schlicker et al., 2008; Schwer et al., 2006).

Sirtuin classification

Sirtuins were first grouped into five major classes (I, II, III, IV and U) (Frye, 2000): classes I-IV each include at least one of the seven sirtuins present in humans; class U includes sirtuins from archaea and bacteria. The last classification of sirtuins included human, *D. melanogaster*, *C. elegans*, three yeast species, rice, plasmodium, leishmania, trypanosoma, and several bacteria and archaea (Frye, 2000). Since then a large number of genome sequences from all kingdoms of life have become available, allowing for a much more comprehensive analysis of sirtuin phylogeny. For instance, it is now possible to analyze sirtuins from all major classes of animals, and thus determine the evolutionary origins of the seven sirtuins encoded in the human genome.

To analyze the evolutionary relationships of sirtuins we searched for all sirtuins present in 77 representative species of animals, plants, bacteria and archaea. We identified sirtuin members in all species we examined with the exception of two red algae and several archaea (Supplementary File 1). At present we cannot rule out that the lack of sirtuin homologs in these species may be due to incomplete sequencing or mistakes in gene predictions, although this seems unlikely in the case of archaea due to the small size of those genomes. Using sequences representing the catalytic core of crystallized human SIRT2, *Archaeoglobus fulgidus* Af1 and *S. cerevisiae* Hst2 sirtuins as templates (Finnin et al., 2001; Min et al., 2001; Zhao et al., 2003b) we aligned 240 sirtuins and used Neighbor Joining methods to construct a phylogenetic tree (Table 1, Fig. 1, Supplementary File 2). The results from this analysis were largely confirmed by a similar analysis using full-length protein sequences (Supplementary File 3). Our analysis mainly confirms the classification of Frye; however, we suggest splitting class Ib sirtuins into two subgroups defined by vertebrate SIRT2 and SIRT3 family members, respectively (Table 1, Fig. 1, Supplementary File 2). It appears likely that the SIRT3 family form a separate subgroup within class Ib sirtuins that originated rather recently, as the SIRT3 group contains only animal species (Fig. 1, Supplementary File 2). Furthermore, the group of "undifferentiated" sirtuins (U), which is not closely related to any vertebrate sirtuin, is comprised of a number of unrelated but clearly defined groups (U1 to U4). U1 is mostly comprised of archaeal sirtuins (grey), while U2 sirtuins are encoded by phylogenetically unrelated single-celled eukaryotes (yellow), such as *Dictyostelium discoideum* (Dd, amoebzoa), *Giardia lamblia* (Gl, excavata) and *Plasmodium falciparum* (Pf, alveolata). U3 and U4 sirtuins are predominantly bacterial (black). Our analysis also establishes that group III is more diverse than previously found, and can be split into three subgroups which we term IIIa, IIIb and IIIc: group IIIa is almost exclusively comprised of animal

sequences, which are predominantly mitochondrially localized, and include human SIRT5; group IIIb sirtuins are predominantly archaeal; and IIIc sirtuins are mostly bacterial. Group IIIa/SIRT5 mammalian sirtuins are related to the IIIc bacterial group, suggesting that IIIa/SIRT5 group sirtuins might be evolutionarily ancient. The majority of sirtuins can be clustered around the seven human sirtuin family members. The only other clusters besides the U1 to U4 group are a group of sirtuins exclusive to fungi (class Ic), which include the *S. cerevisiae* Hst3 and Hst4 sirtuin family members. The Ic sirtuin family likely arose early during fungal evolution, as such sirtuins were found in all fungal species we examined, including members of ascomycota, basidiomycota and microsporidia (Table 1, Fig. 1, Supplementary File 2).

Evolutionary considerations

According to Baldauf (2003) eukaryotes can be categorized into 8 major phylogenetic groups: opisthokonts includes animals, fungi and choanoflagellates; plants make up another major group; and the remaining 6 groups are mostly single celled organisms, including amoebzoa (containing *Dictyostelium discoideum*) and discicristates (containing *Leishmania*). To investigate the evolutionary origins of the seven groups defined by human sirtuins we commenced our analysis by looking for sirtuins within the group of the opisthokonts. This group includes animals, fungi and choanoflagellates such as the recently sequenced *Monosiga brevicollis* (Mbr, light blue) (King et al., 2008). *Monosiga* sirtuins can be clearly found in the SIRT1, SIRT2 and SIRT4 and SIRT7 group, although a second class IV *Monosiga* sirtuin cannot be clearly assigned to the SIRT6 or the SIRT7 group (Fig. 1, Table 1). With very few exceptions fungal sirtuins (orange) cluster within class I sirtuins. Besides the class Ic group, which is fungus-specific, fungal sirtuins are also found within the SIRT1 and the SIRT2 groups. Interestingly, we also found a single fungal representative in class IV sirtuins that cannot clearly be grouped with SIRT6 or SIRT7 like sequences. Furthermore, there is a single fungal representative each within the SIRT4 and the SIRT5 group. Most fungi analyzed contain 5 sirtuins, with the exception of *S. pombe* (encoding 3 sirtuins), and *Encephalitozoon cuniculi* (Katinka et al., 2001) that encodes only one sirtuin of the fungus-specific class. Thus sirtuins encoded by fungi and Choanoflagellates are represented in all groups except for SIRT3, arguing for an early radiation of sirtuins in the evolution of Opisthokonts (Fig. 1, Table 1).

We next looked at the plants, a further major group of eukaryotes that includes vascular plants, mosses, and red and green algae. Surprisingly, we found that the moss *Physcomitrella patens* (Rensing et al., 2008) encodes sirtuin SIRT4- to SIRT7-like sequences, while only SIRT4 and SIRT6 were found in the angiosperms we analyzed (Fig. 1, Table 1). Interestingly, one green alga, *Ostreococcus lucimarinus*, encodes a SIRT2 homolog, which appears to be lost in higher plants. Surprisingly, we were not able to find any sirtuins in red algae, although at present we cannot be sure whether this is due to incomplete sequence information. Members of five further groups of eukaryotes, namely amoebzoa [*Dictyostelium discoideum* (Eichinger et al., 2005; Gardner et al., 2002)], alveolata [*Plasmodium falciparum* (Gardner et al., 2002)], heterokonta [*Phaeodactylum tricornutum* (Bowler et al., 2008)], excavata (*Giardia lamblia*) and discicristata, (*Leishmania infantum*) contain divergent sirtuins that cannot be firmly assigned phylogenetically, as well as SIRT2, SIRT4 and SIRT6 family members (Fig. 1, Table 1).

Fig. 1. Unrooted phylogenetic tree of all aligned sirtuin sequences. The tree was constructed using SplitsTree 4 (Huson and Bryant, 2006). Classes defined by Frye (2000) are shown in black, classes added in this review are shown in red. Branches for which bootstrap values are shown are also in red. The SplitsTree file with all bootstrap values can be downloaded as Supplementary File 2.

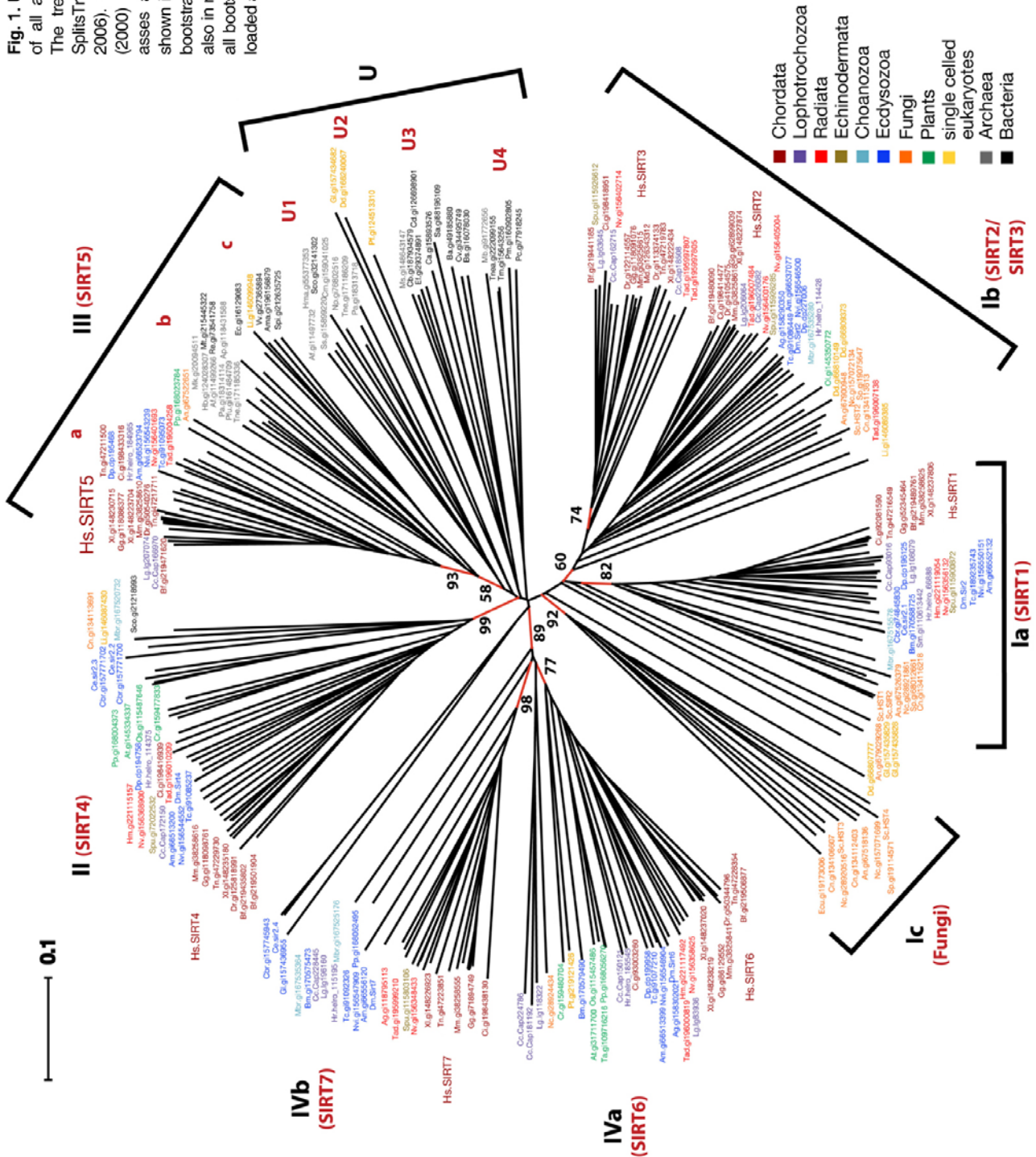


Table 1. Sirtuin complement of analyzed eukaryotes. Numbers indicate one or more SIRT1-SIRT7 related sirtuins, question marks indicate sirtuins that could not be assigned to groups defined by mammalian sirtuins. The number of question marks indicates the number of unassigned sirtuins. Question marks between the SIRT6 and SIRT7 column signify class IV sirtuins that could not clearly be placed into the SIRT6 or SIRT7 groups. Colors correspond to the classification described in Fig. 1. "F" signifies class Ic sirtuins that are specific to Fungi. 1) to 5) indicates various phylogenetic groups: 1) urochordata; 2) crustacea; 3) basidiomycota; 4) microsporidia; 5) bryophyta

				Ia	Ib	II	III	IVa	IVb
opisthokonta	chordata vertebrata	Dr	<i>Danio rerio</i>	zebra fish	2	3	4	5	6
		Gg	<i>Gallus Gallus</i>	chicken	1	2	3	4	5 6 7
		Hs	<i>Homo sapiens</i>	human	1	2	3	4	5 6 7
		Mm	<i>Mus musculus</i>	mouse	1	2	3	4	5 6 7
		Tn	<i>Tetraodon nigroviridis</i>	pufferfish	1		3	4	5 6 7
		Xl	<i>Xenopus laevis</i>	frog	1	2	3	4	5 6 7
		1) Bf	<i>Branchiostoma floridae</i>	lancelet	1	2	3	4	5 6
	lophotro chozoa	Ci	<i>Ciona intestinalis</i>	sea squirt	1	2	3	4	5 6 7
		Hr	<i>Helobdella robusta</i>	leech	1	2		4	5 6 7
		Cc	<i>Capitella capitata</i>		1	2	3	4	5 6 7
	radiata	Lg	<i>Lotia gigantea</i>	snail	1	2	3		5 6 7
		Hm	<i>Hydra magnipapillata</i>		1			4	6
		Nv	<i>Nematostella vectensis</i>	sea anemone	1	2	3	4	5 6 7
	echinod.	Tad	<i>Trichoplax adhaerens</i>			2	3	4	5 6 7
		Spu	<i>Strongylocent. purpuratus</i>	sea urchin	1	2	3	4	7
	choanoz.	Mbr	<i>Monosiga brevicollis</i>		1	2		4	? 7
	ecdysozoa insecta	Ag	<i>Anopheles gambiae</i>	mosquito		2			6 7
		Am	<i>Apis mellifera</i>	honey bee	1	2		4	5 6 7
		Dm	<i>Drosophila melanogaster</i>	fruit fly	1	2		4	6 7
		Nvi	<i>Nasonia vitripennis</i>	wasp	1	2		4	5 6 7
		Tc	<i>Tribolium castaneum</i>	beetle	1	2		4	5 6 7
		2) Dp	<i>Daphnia pulex</i>	water flea	1	2		4	5 6
	nema- toda	Bm	<i>Brugia malayi</i>		1				6 ? ?
		Cbr	<i>Caenorhabditis briggsae</i>		1		4		? ?
		Ce	<i>Caenorhabditis elegans</i>		1		4		? ?
	fungi asco- mycota	Sp	<i>Schizosacch. pombe</i>	fission yeast	1	2			F
		An	<i>Aspergillus nidulans</i>		1	2		5	F ?
		Nc	<i>Neurospora crassa</i>		1	2			? F
		Sc	<i>Saccharomyces cerevisiae</i>	budding yeast	1	2			F
		3) Cn	<i>Cryptococcus neoformans</i>		1	2		4	F
		4) Ecu	<i>Encephalitozoon cuniculi</i>						F
		Cr	<i>Chlamydomonas reinhardtii</i>	green algae				4	
plants	land plants angio- sperm	Oi	<i>Ostreococcus lucimarinus</i>	green algae	2				
		At	<i>Arabidopsis thaliana</i>	thale cress				4	6
		Os	<i>Oryza sativa</i>	rice				4	6
		Ta	<i>Triticum aestivum</i>	wheat					6
		5) Pp	<i>Physcomitrella patens</i>	moss				4	5 6 7
	rhodo- phyta		<i>Guillardia theta</i>	red algae					no Sirtuins
			<i>Hemiselmis andersenii</i>	red algae					no Sirtuins
	amoebzoa	Dd	<i>Dictyostelium discoideum</i>		2				??
	alveolata	Pf	<i>Plasmodium falciparum</i>						?
	heterokonta	Pt	<i>Phaeodactylum tricornutum</i>					6	
	excavata	Gl	<i>Giardia lamblia</i>						???
	discicristata	Li	<i>Leishmania infantum</i>		2		4		?

We next searched for sirtuins encoded in animals with radial symmetry (radiata), which include examples of cnidaria (corals, sea anemones and jellyfish) such as the starlet sea anemone *Nematostella vectensis* and *Hydra magnipapillata*. Amongst this group of animals, the placozoan *Trichoplax adhaerens* is a representative of a basal eumetazoan lineage (all animal

clades except sponges) that diverged before the separation of cnidarians and bilaterians (Srivastava et al., 2008). *Trichoplax* is comprised of a flat disc of cells with two epithelial layers sandwiching a layer of multinucleate fiber cells. Within this species we could find homologs of all human sirtuin groups except for SIRT1. Thus relatives of all seven sirtuin groups are already

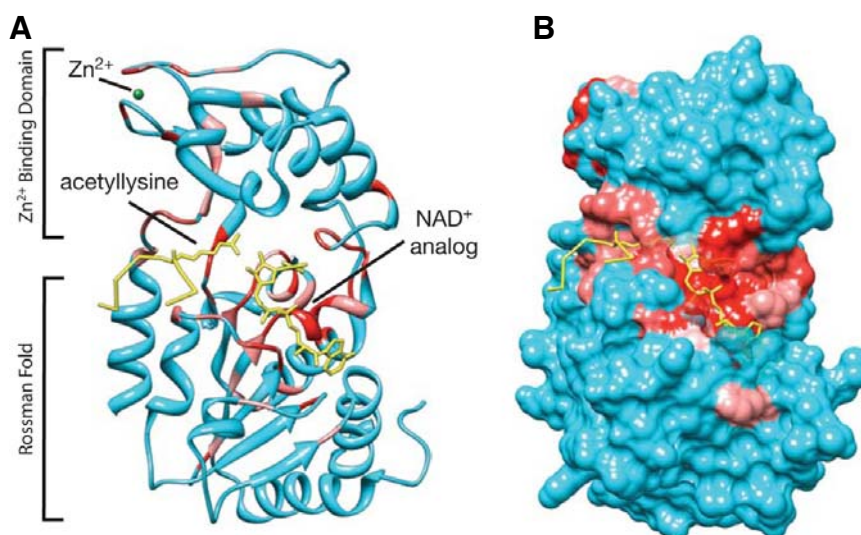


Fig. 2. Structural context of conserved residues. Structure of budding yeast Hst2p catalytic domain in complex with acetyllysine histone H4 peptide (yellow) and a non-hydrolyzable NAD⁺ analogue (yellow) (Zhao et al., 2004). Amino acids 4 to 204 and 213 to 287 of the structure were used. Amino acids 205 to 212 (WLREKITT) were excluded because they are unique to Hst2p and not conserved in any other sirtuin. Conserved residues were visualized using Chimera software (Meng et al., 2006; Pettersen et al., 2004); highly conserved residues are shown in red. A rotational 360° view of the structure is shown in Supplementary File 5 (ribbon view, Fig. 1A) and Supplementary File 6 (surface view, Fig. 1B).

present in animals with radial symmetry, and indeed all of them can be found in *Nematostella*. The absence of a SIRT1 homolog in *Trichoplax* and of several sirtuins in *Hydra* could also be due to incomplete sequence information. Nevertheless, our analysis clearly indicates that representatives of all seven sirtuin groups were present in the common ancestor of all animals.

The two most important non-vertebrate animal models are the nematode *C. elegans* and the fruit fly *D. melanogaster*. Insects, forming part of the larger group of arthropods, and nematodes are classified as members of the ecdysozoa (Der Ou et al., 2007). Consistent with this grouping, we find that all ecdysozoan species we analyzed failed to encode a sirtuin of the SIRT3 group, which we deduce must have been lost early in the evolution of ecdysozoa. Further, the focused analysis of nematode sequences provides evidence for extensive loss of sirtuins at an early evolutionary stage. Fully sequenced *C. elegans* and *C. briggsae* genomes (Stein et al., 2003) contain clear SIRT1 and SIRT4 homologs, while an additional divergent sirtuin in both *Caenorhabditis* species does not cluster with any other sirtuin groups. Interestingly, another nematode *Brugia malayi* (Ghedini et al., 2007) does not contain a SIRT4 homolog, although clear SIRT1, SIRT6 and SIRT7 homologs are encoded. Within arthropods, several insects including the honey bee *Apis mellifera* (Solignac et al., 2007), the parasitoid wasp *Nasonia vitripennis*, and the red flour beetle *Tribolium castaneum* (Richards et al., 2008) contain homologs of all sirtuins except SIRT3, while the nearly completely sequenced *Drosophila melanogaster* genome additionally lacks a SIRT5 homolog, and the mosquito *Anopheles gambiae* encodes only three sirtuins. Thus, the loss of specific sirtuin groups is characteristic of nematodes and arthropod lineages.

Structural features and biochemical function

A number of sirtuins from different organisms have been crystallized (Avalos et al., 2002; Chang et al., 2002; Finnin et al., 2001; Min et al., 2001; Zhao et al., 2003a; 2003b). They all consist of a highly conserved catalytic core of approximately 250 amino acids, composed of a NAD⁺-binding Rossmann fold domain, and a Zn²⁺-binding domain containing four highly conserved cysteine residues (Fig. 2). The catalytic site is situated inside a hydrophobic channel formed at the interface of the two domains, wherein the end of the acetyllysine side chain is ar-

ranged in close proximity to the nicotinamide ribose of NAD⁺ (Fig. 2A, Supplementary File 5). In contrast to class I, II and IV histone deacetylases where zinc participates in catalysis to produce free acetate and deacetylated lysine (Holbert and Marmorstein, 2005), sirtuins do not use zinc in their catalytic center, but it rather has a structural role. Sirtuins transfer the acetyl moiety from the ε-amino group of lysine to the nicotinamide ribose of NAD⁺ (Fig. 3). The sirtuin reaction is thought to proceed in two stages: firstly the acetyl oxygen replaces nicotinamide either by an S_N1 or more likely an S_N2 mechanism (Hu et al., 2008) to produce an O-alkylamide intermediate and nicotinamide product; in the second step the acetyl group is transferred to ADP-ribose to form O-acetyl-ADP-ribose and deacetylated lysine (Fig. 3). O-acetyl-ADP-ribose is a metabolite uniquely generated by sirtuin deacetylases (Imai et al., 2000; Landry et al., 2000; Sauve et al., 2001; Smith et al., 2000; Tanner et al., 2000; Tanny and Moazed, 2001).

Numerous studies have found that some sirtuins also possess ADP-ribosyl transferase activity in addition to their protein deacetylase activity, amongst them are yeast Sir2p, trypanosoma Sir2, and mammalian SIRT4 and SIRT6 (Haigis et al., 2006; Kowieski et al., 2008; Liszt et al., 2005; Tanny et al., 1999; Tsang and Escalante-Semerena, 1998). For SIRT4, only ADP-ribosylation but no deacetylation activity has so far been reported (Ahuja et al., 2007; Haigis et al., 2006). However, a recent study using yeast Sir2p and Hst1p, as well as several mammalian sirtuins including SIRT4, has questioned the physiological significance of sirtuin-catalyzed ADP-ribosylation. Du et al. found that the ADP-ribosyl transferase activity of sirtuins is far weaker than their protein deacetylation activity and several orders of magnitude below that of the bacterial ADP-ribosyl transferase, diphtheria toxin. ADP-ribosylation may therefore be an insignificant side reaction (Du et al., 2009).

Generating a multiple sequence alignment (Supplementary File 4) allowed us to visualize the level of conservation of amino acids on the published budding yeast Hst2 structure. It is apparent that almost all highly conserved residues (red) are involved in the formation of the catalytic channel, the binding of NAD⁺, and in the coordination of the acetyllysine (Fig. 2B, Supplementary File 6). Apart from the amino acids forming the catalytic channel, the most highly conserved residues are four structurally important cysteines that coordinate Zn²⁺ binding. Although no clear target consensus sequences have yet been

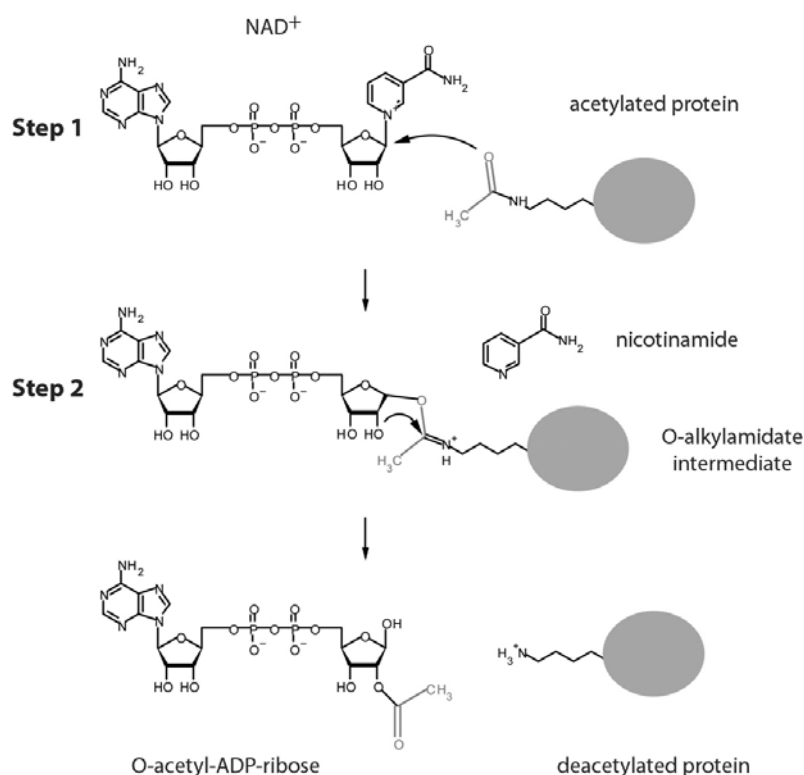


Fig. 3. Deacetylation mechanism catalyzed by sirtuins. Sirtuin-mediated deacetylation proceeds in two steps. In the first step nicotinamide is cleaved, yielding an O-alkylamidate intermediate. In the second step the nicotinamide ribose 2'OH group attacks the intermediate, yielding deacetylated lysine and O-acetyl-ADP-ribose.

identified in sirtuin substrates, it is possible that sirtuins recognize their targets through interactions that lie outside the catalytic domain (Blander et al., 2005; Khan and Lewis, 2005; Mead et al., 2007). While the majority of sirtuin proteins consist solely of a catalytic domain, the class Ia subgroup that includes the human SIRT1 homolog of budding yeast Sir2p, also contain extensive N- and C-terminal domains (Frye, 2000). It is interesting to speculate that these domains are needed to interact with binding partners, or for conferring specificity for substrate recognition.

CONCLUSION

Despite the recent explosion in the number of reports on sirtuins there are still substantial gaps in our understanding of sirtuin function. Our evolutionary analysis provides clear evidence that all seven sirtuin families are ancient in animal evolution. Thus, a clearer understanding of the function of sirtuins may benefit from the analysis of simple organisms. For instance, the use of RNAi technology in the cnidarian *Nematostella vectensis*, may aid in elucidating the key functions related to individual sirtuins (Pankow and Bamberger, 2007). Given that sirtuins have been selectively and extensively lost during evolution, especially in insects, nematodes and plants, it appears likely that the loss of individual sirtuins might be compensated for redundant functions conferred by remaining sirtuin family members. This might indeed explain the relatively weak phenotypes associated with the reported murine single sirtuin knockouts, that all permit development into adult mice. Furthermore, functional redundancy between sirtuins may account for the failure to confirm the physiological importance of sirtuin-mediated deacetylation events of known *in vitro* substrates. Thus, to understand the core functions of sirtuins it will be necessary to analyze strains with mutations in multiple sirtuins

using genetically tractable model organisms containing few sirtuin homologs, such as fruit flies, nematodes or *Arabidopsis*. Additionally, very little is known about the mechanism(s) of substrate recognition by sirtuins, and whether this requires additional proteins. Budding yeast Sir2p is known to be part of different complexes with distinct biological functions (Aparicio et al., 1991; Shou et al., 1999; Straight et al., 1999). It will therefore be interesting to assess whether this is a common theme in sirtuin regulation. Again, a phylogenetic approach might provide helpful insights. For instance, while sirtuin conservation is highest around the catalytic center, more extensive modeling of individual sirtuin classes combined with structural studies might identify conserved surface residues likely to be important for mediating interactions to provide either substrate specificity or binding specificity for essential protein cofactors. Given the increasing evidence of sirtuin-mediated protection from aging and aging-related neurodegenerative disease, the development of compounds that specifically activate or inhibit individual sirtuin members could be aided by comparative phylogenetic and structural analyses.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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