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Review

Sirtuins of parasitic protozoa: In search of function(s)

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ABSTRACT

The SIR2 family of NAD$^+$-dependent protein deacetylases, collectively called sirtuins, has been of central interest due to their proposed roles in life-span regulation and ageing. Sirtuins are one group of environment sensors of a cell interpreting external information and orchestrating internal responses at the sub-cellular level, through participation in gene regulation mechanisms. Remarkably conserved across all kingdoms of life SIR2 proteins in several protozoan parasites appear to have both conserved and intriguing unique functions. This review summarises our current knowledge of the members of the sirtuin families in Apicomplexa, including *Plasmodium*, and other protozoan parasites such as *Trypanosoma* and *Leishmania*. The wide diversity of processes regulated by SIR2 proteins makes them targets worthy of exploitation in anti-parasitic therapies.

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1. The sirtuin family – introduction

Recently a wealth of data has emerged that implicates protein acetylation as a major (and frequently overlooked) cellular mechanism to control protein activity through post-translational modification. Protein acetylation is well characterised in the case of chromatin (*e.g.* histones), and acetylated proteins are highly abundant in the nucleus with targets including chromatin modifying enzyme complexes affecting all major nuclear processes. Furthermore, protein acetylation appears to play a central role in many cytoplasmic events, such as protein folding, signal transduction, cell cycle control and cytoskeletal regulation. The acetylation status of a target protein is determined by the action of protein acetylases and deacetylases – a large family of diverse proteins that includes the sirtuin proteins which are the focus of this review.

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The silent-information regulator 2 (SIR2)-like family of nicotinamide nucleotide dinucleotide (NAD^+)-dependent protein deacetylases, commonly called sirtuins [1,2], are highly conserved from archaea to higher eukaryotes, and are arguably most renowned for their role in longevity. Sirtuins have a wide scope of cellular actions and have been in the spotlight of ageing, life-span and metabolism studies and are linked to a number of diseases, including Alzheimer's disease, obesity, type II diabetes, neurodegenerative disorders and cancer (recent reviews see *e.g.* [3–7]). At the cellular level sirtuins collectively participate in many cell activities including but not restricted to gene silencing, cell cycle progression, chromosome segregation, microtubule organisation, protein aggregates transport, genome stability, DNA repair, apoptosis, autophagy (for reviews see for instance [8,9]).

Since sirtuins are central to proper cell functioning and proliferative life span, their role in pathogenic organisms such as those contained within the Apicomplexa is intriguing. This review will summarise the recent advances in the study of sirtuins in a number of parasitic protozoa of medical and veterinary importance, comparing these findings with those from other organisms where they have been described in some detail. Sirtuins in parasitic protozoa have both the canonical and atypical activities that contribute to both conserved and apparently unique functions. The biological implications and available sirtuin-targeting drugs, which could be used in treatments of parasitic disease are also discussed.

2. The phylogenetic distribution of sirtuins

Initial phylogenetic analysis classified 60 sirtuins into five main types based on the different sirtuin domains of the 7 human sirtuins (hSIRT1–7) [10] with type IV subdivided into IVa and IVb, and type I into a, b and c. A very recent phylogenetic analysis of 240 sirtuins confirmed these classifications and proposed further subdivisions of Class III (a, b and c) and split the undifferentiated (U) category into U1–U4 branches [11]. Our more extensive phylogenetic tree is based on Neighbour-Joining (NJ) method, for more than 700 sirtuin sequences from 529 organisms including the parasitic protozoa (Fig. 1A and see Suppl. Table 1 for a list of sequences originating from parasite genomes). The different protozoan parasite genomes are predicted to contain one or multiple sirtuin genes (see below). The global tree greatly resembles the earlier analyses with class I–IV (colours: teal, pink, red and purple, respectively), and class U (blue) (subclasses of the U class are not yet supported). Sequence similarity networks (SSN; Fig. 1B) incorporate not only aligned sequences but may be combined with experimental data such as substrate specificity and subcellular location to derive their graphical output. This provides a more practical visualisation of functional relationships between members of a protein family and emphasises the diverged nature of the unclassified members. Fig. 1B represents putative sirtuins of parasitic protozoa and their relative distances obtained from blasting the parasitic protozoa sequences against all the sequences used in defining the canonical sirtuin domain (PFAM ID: PF02146; http://pfam.sanger.ac.uk/). Parasite sirtuins are distributed in all of the phylogenetically defined sirtuin classes. Class I sirtuins (coloured green) are found only in Sarcomastigophora (includes the Trypansomatids) and not in Apicomplexa (see Table 1 for details). Although a *Trichomonas vaginalis* sirtuin (TVAG_146810) and a *Giardia lamblia* sirtuin (GL50803_11676) are relatively distant from the remaining parasitic protozoan sirtuins in that class. Class II (coloured in pink) contains one of the trypansomatid sirtuins from the three *Leishmania* species (LmjF23.1210, LbrM23_V2.1310, LinJ23_V3.1450) and *Trypanosoma brucei* (Tb927.8.3140; circled in pink in Fig. 1B). Most apicomplexans possess 2 sirtuins: SIR2A and SIR2B (triangles). SIR2A sirtuin domains can be assigned to class III (coloured red) with moderate support (as previously described in *e.g.* [10,12,13]), or class U (hence coloured red circled in blue). Apicomplexa SIR2B sirtuin domains all cluster very well with class IV sirtuins (purple box) together with human SIRT6 and SIRT7 (human SIRT1–7; Fig. 1B diamonds). Perhaps unsurprisingly, a sirtuin of the ancient protist *G. lamblia* (GL50803_6942) appears to be the most distant example of protozoan sirtuins analysed and groups with putative sirtuins of archaebacteria possibly reflecting the proposed ancient origin of *Giardia*. Another *G. lamblia* SIR2 (GL50803_16569) forms an outlying cluster (circled in black), with the single class U SIR2 predicted in the *Cryptosporidium* spp. genomes (triangles). Human sirtuins typify classes I–IV. Yeast SIR2s as well as hSIRT1–4 all cluster into class I (in agreement with the original classification [10]).

Table 1 lists parasitic protozoa shown in Fig. 1B, and number and classification of sirtuins found in their genomes. As previously mentioned Apicomplexa possess only 1–2 SIRs (based on available sequence data; see data source column, Table 1), whereas the remaining characterised parasitic protozoan lineages apparently have more. For instance based on the current genome assembly (scaffold genome 01/2007 [14]; search performed on TrichDB v1.3 on 08/2011) of *T. vaginalis* as many as 11 distinct sirtuins are found in the genome (TrichDB, blast analysis using *S. cerevisiae* SIR2/YDL042C as query) although this prediction may yet reflect the incomplete nature of the genome sequence and its annotation. In summary sirtuin domains of parasitic protozoa are widely dispersed across the sirtuin classes. Nevertheless the sirtuin domain is structurally highly conserved as shown below indicating general functional conservation, *i.e.* involvement in NAD^+-dependent protein acetylation and/or ADP ribosylation.

3. Structure

Sirtuin family members share a catalytic domain that allows the majority of sirtuins to function as NAD^+-dependent protein deacetylases. However the same domain generally acts as ADP-ribosyltransferase in some cases exclusively so and this specialised type of sirtuin appears currently restricted to class II sirtuins (which includes bacterial and human SIRT4, see Fig. 1A). A typical sirtuin (shown in Fig. 2A, see also Fig. 2C) largely consists of a sirtuin catalytic domain, which in turn comprises both a NAD-binding and acetyl-lysine-binding domain (red boxes in Fig. 2A), as well as a variable zinc ion-binding domain implicated in substrate specificity of different sirtuin proteins. The PFAM database defines the canonical sirtuin domain (PFAM PF02146) which consists of several highly conserved subdomains stretching over 181 amino acids. Sirtuin domains of parasitic protozoa (database IDs in Table 1) conform to the canonical sirtuin domain, including several of the highly conserved regions (Fig. 2A black boxes). A number of residues are perfectly conserved within Apicomplexa (asterisked) which is further strengthened by MEME motif analysis (http://meme.sdsc.edu; Fig. 2B) of all Apicomplexa sirtuins, which demonstrated localised high conservation within the sirtuin domain. The Class IV type SIR2Bs apicomplexan SIR2Bs (*Plasmodium*, *Toxoplasma*, *Neospora*, *Theileria*, *Babesia* and *Eimeria*) all contain several extra apparently SIR2B-specific motifs in addition to the canonical sirtuin domain, with the exception of *Eimeria tenella* SIR2B (Eth_SIR2B) which appears incomplete in sequence. The functional significance of SIR2B-specific motifs awaits characterisation. The sirtuin domain flanking regions typically determine the functional context such as subcellular localisation and protein–protein interactions modulating substrate range [15]. For instance, mammalian SIRT1 exhibits different modes of nucleo-cytoplasmic shuttling depending on tissue or cell type. Two N-terminal nuclear localisation signals (NLS) and two nuclear export signals (NES) functionally identified in