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Review

Mitochondrial sirtuins

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ABSTRACT

Sirtuins have emerged as important proteins in aging, stress resistance and metabolic regulation. Three sirtuins, SIRT3, 4 and 5, are located within the mitochondrial matrix. SIRT3 and SIRT5 are NAD^+-dependent deacetylases that remove acetyl groups from acetyllysine-modified proteins and yield 2′-O-acetyl-ADP-ribose and nicotinamide. SIRT4 can transfer the ADP-ribose group from NAD^+ onto acceptor proteins. Recent findings reveal that a large fraction of mitochondrial proteins are acetylated and that mitochondrial protein acetylation is modulated by nutritional status. This and the identification of targets for SIRT3, 4 and 5 support the model that mitochondrial sirtuins are metabolic sensors that modulate the activity of metabolic enzymes via protein deacetylation or mono-ADP-ribosylation. Here, we review and discuss recent progress in the study of mitochondrial sirtuins and their targets.

1. Introduction

Mitochondria are double-membrane bound organelles that play a central role in energy production. Increasing evidence suggests a key role for mitochondrial dysfunction in diabetes, aging, neurodegenerative disorders, and cancer [1–3]. Many mitochondrial proteins are multiply acetylated [4,5] and mitochondrial protein acetylation levels are modulated during calorie restriction and fasting [4,6]. It has been well established that protein acetylation is dynamic and regulated by the competing enzymatic activities of protein acetyltransferases and protein deacetylases.

Histone/protein deacetylases are enzymes that catalyze the removal of acetyl groups from the ε-amino group of lysine residues and are classified into three groups. Sirtuins, the class III histone deacetylases, are homologous to the yeast transcriptional repressor, Sir2. Unlike previously characterized class I and II histone deacetylases, which catalyze the simple hydrolysis of acetyllysine [7,8], Sir2 deacetylates lysine residues in a novel chemical reaction that consumes nicotinamide adenine dinucleotide (NAD^+) and generates nicotinamide, O-acetyl-ADP-ribose (OAADRr), and the deacetylated substrate [9–11]. Seven sirtuins have been identified in the human genome [12,13]. They share a conserved Sir2 catalytic core domain and exhibit variable amino- and carboxyl-terminal extensions that contribute to their unique subcellular localization and may also regulate their catalytic activity. The subcellular distribution, substrate specificity, and cellular functions of sirtuins are quite diverse. SIRT1 is found in the nucleus, where it functions as a transcriptional repressor via histone deacetylation. SIRT1 also regulates transcription by modifying the acetylation levels of transcription factors, such as MyoD, FOXO, p53, and NF-κB [14–20]. The SIRT2 protein is found in the cytoplasm, where it associates with microtubules and deacetylates lysine 40 of α-tubulin [21]. SIRT6 functions primarily as a histone H3K9 deacetylase and regulates telomeric chromatin [22]. SIRT7 is localized in the nucleolus and functions as a positive regulator of RNA polymerase I transcription [23].

Interestingly, three sirtuins, SIRT3, SIRT4, and SIRT5, are located in mitochondria (Table 1). Here, we review the emerging roles for each of the mitochondrial sirtuins, including their known substrates and functions, and discuss their possible biological roles under normal and pathological conditions.

2. Reversible mitochondrial protein acetylation

A novel experimental approach combining immuno-affinity purification of peptides carrying acetylated lysine residues after proteolytic digestion of mitochondrial extracts and mass spectroscopic analysis has led to the identification of a large number of mitochondrial acetylated proteins. At least 20% of mitochondrial proteins are acetylated. An early proteomic survey identified 277 acetylation sites on 133 proteins in liver mitochondria from fed and fasted mouse [4]. Proteins from all major metabolic pathways, including TCA cycle, oxidative phosphorylation, β-oxidation and the urea cycle were represented [4]. Acetylated lysine residues are also

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found in mitochondrial channel proteins and in proteins involved in amino acid, carbohydrate and nucleotide metabolism [4]. The observation that some of these acetylated proteins are unique to fed, fasted or calorie-restricted conditions in mice strongly supports a regulatory role to acetylation in mitochondria [4,6].

Mitochondria are originally derived from endosymbiotic prokaryotes and have many features in common with prokaryotes. Similar to mitochondria, *E. coli* contains abundant lysine-acetylated proteins and the majority (53%) of its lysine-acetylated proteins are metabolic enzymes [24]. Significant changes in the *E. coli* lysine acetylation profile are seen following exposure to hypoxia but not starvation [24], supporting the model that reversible protein acetylation allows cells to adjust to a changing environment.

A key unresolved question is where do mitochondrial proteins become acetylated? A mitochondrial protein acetyltransferase has not yet been identified but ATP synthase F_o subunit 8, one of 15 proteins encoded by mitochondrial DNA, is acetylated, implying that the acetylation reaction can occur in mitochondria for some proteins [4]. A bacterial acetyltransferase, Pat, has been identified and uses acetyl-CoA to acetylate the bacterial enzyme acetyl-CoA synthase (AceCS) [25,26]. CobB, a bacterial sirtuin homolog, is responsible for the deacetylation of AceCS. The C-terminal 95 amino acids of Pat show homology to the eukaryotic GCN5 acetyltransferase superfamily [26]. Orthologs of Pat are also identified in the haloarchaeon *Haloferax volcanii* and the archaeon *Sulfolobus solfataricus* [27,28]. However, no Pat orthologs have been identified in mammalian cells and the identity of the mitochondrial acetyltransferase in mammals remains unknown. It is also possible that some protein acetylation reactions occur in a non-enzymatic manner [29,30].

Interestingly, acetylation motifs for cytosolic and nuclear proteins are similar, but distinct from acetylated motifs in mitochondrial proteins [4]. In the cytosol and the nucleus, asparagine is favored at the −1 position and histidine at the +1 position, where 0 denotes the lysine [4]. In the mitochondria or *E. coli*, histidine or tyrosine is favored at the +1 position [4,24]. This substrate specificity difference may suggest that the mechanism of acetylation of mitochondrial proteins is distinct from cytosolic and nuclear proteins.

Whether all mitochondrial proteins are acetylated after import into mitochondria and whether the process is mediated by a mitochondrial acetyltransferase or via non-enzymatic acetylation are important question that will need to be addressed in the future to fully understand the biology of protein acetylation in mitochondria.

The proteomic survey of mitochondria acetylation is still not complete. Due to the limited sensitivity of mass spectrometry, acetylated lysine of low abundance may be missed. A recent approach added a purification step of acetylated lysine peptides by isoelectric focusing to increase the number of identified acetylated peptides [5]. Also, different anti-acetyllysine antibodies react differentially and may immunoprecipitate different acetylated lysine peptides.

As discussed in detail below, mitochondria contain NAD^+-dependent protein deacetylase activity and three sirtuins, SIRT3, 4 and 5 have been identified in the mitochondrial matrix. In contrast to other cellular compartments, no NAD^+-independent protein deacetylase activity is detected in mitochondria when using histone peptide as substrate [31]. This suggests that sirtuins may be the major protein deacetylases within mitochondria.

Interestingly, knockout mice lacking SIRT4 and SIRT5 show no apparent change in the acetylation level of mitochondrial protein. This is in contrast to mitochondria isolated from mice lacking SIRT3 which show a dramatic increase of mitochondria protein acetylation [32]. These findings suggest that many mitochondrial proteins are subject to deacetylation by SIRT3 while the deacetylase activity of SIRT4 and SIRT5 might be more restricted. Interestingly also, the acetylation of several proteins appeared unaffected by the absence of SIRT3, SIRT4 or SIRT5, suggesting that a number of acetylation sites in mitochondrial proteins may not be regulated by sirtuins. It is also possible that other deacetylase activities exist within mitochondria and regulate these acetylation sites.

3. SIRT3

SIRT3 is a soluble protein located in the mitochondrial matrix [31–33]. The mitochondrial-targeting peptide of human SIRT3 at the N-terminus of the precursor protein is cleaved off after import into mitochondria [31]. The cleavage can be catalyzed by mitochondrial processing peptidase (MPP) *in vitro*. Two arginine residues, Arg 99 and Arg 100, are essential for cleavage [31] and are conserved among many species [34]. Full protein deacetylase activity of human SIRT3 is obtained only after proteolytic processing [31]. Similarly, unprocessed mouse SIRT3 does not show deacetylase activity and only becomes enzymatically active after the removal of the N-terminal sequence [35].

The originally described mouse SIRT3 cDNA lacked an N-terminal mitochondrial-targeting signal (short SIRT3) [36,37] and some controversy arose about the subcellular localization of mouse SIRT3. However, a recently cloned SIRT3 cDNA encodes a longer protein (long SIRT3) which includes an N-terminal mitochondrial-targeting signal similar to the one present in human SIRT3 [38,39]. Importantly, this cDNA encodes a mitochondrial protein, long SIRT3, which is processed to a mature protein with the same exact size as endogenous mouse SIRT3. The size of the originally reported SIRT3 short form is smaller than endogenous SIRT3 in primary mouse hepatocytes [38]. Although mRNAs for both the short and long forms of SIRT3 are present *in vivo* [37–39], the physiological function and the nature of the existence of SIRT3 short form protein are unclear and need to be further investigated.

Additional controversy has arisen on the possibility that SIRT3 might also be present in the nucleus [40–42] (see [43] for a recent thorough discussion of this issue). However, stringent fractionation studies show that both endogenous and overexpressed human SIRT3 are localized in mitochondria [34]. Endogenous mouse SIRT3 is also found exclusively in mitochondria [32]. Furthermore, both mouse and human SIRT3 contain a cleavable N-terminal mitochondrial-targeting presequence [31,39]. These observations do not completely exclude the possibility that a small fraction of SIRT3 might be nuclear but it should be emphasized that the bulk of SIRT3, both mouse and human, is localized in the mitochondrion.

Table 1
Characteristics of mitochondrial sirtuins.

	Sub-mitochondrial localization	Mouse tissue expression	Substrate		Interacting protein	References
			Deacetylation	ADP-ribosylation		
SIRT3	Matrix	High in kidney, heart, liver, brain, and brown adipose tissue	AceCS2, GDH, Ku70, ICDH2, and electron transport chain Complex I (including NDUFA9)		FOXO3a, ATP5a, and Hsp70	[31,40,44,47,100,101]
SIRT4	Matrix	High in kidney, heart, liver, and brain		GDH	IDE, ANT2/3, and SIRT3	[53,54]
SIRT5	Matrix and intermembrane space	High in kidney, heart, liver, brain, and skeletal muscle	CPS1 cytochrome *C*			[48,49]