

At the crossroad of lifespan, calorie restriction, chromatin and disease

Meeting on sirtuins

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Longevity, lifespan, cancer, cellular transformation, energy, calorie restriction, diabetes... what can tie together such a diversity of hot topics in biomedical research? Emerging findings suggest that the answer lies in understanding the functions of the recently discovered family of proteins known as Sirtuins. Barcelona hosted the first scientific meeting completely focused on these evolutionary conserved protein deacetylases, bringing together experts in the biochemistry to cellular biology, mice models, drug targeting and pathophysiology of these molecules. Their work, summarized here, establishes the Sirtuins as major players in cellular homeostasis and human diseases that act through a whole range of biochemical substrates and physiological processes. Undoubtedly, this is an increasingly expanding field that it is here to stay and growth.

The Active Involvement of Sirtuins in Chromatin Regulation

Histone acetylation/decacetylation is a well-characterized mechanism of chromatin regulation. Since sirtuins were discovered as a class of deacetylases ten years ago, there has been prominent interest in defining their function in this process. Of all seven sirtuins only SIRT1, 6 and 7 seem to be restricted to the nucleus, although SIRT1 can also be detected in the cytoplasm in certain cellular conditions (see Fig. 1). In contrast, other sirtuins such as cytoplasmic SIRT2 and mitochondrial SIRT3 seem to localize to the nucleus

either in a small subpopulation (SIRT3) or in certain stages of the cell cycle (such as SIRT2). Despite the long-standing link between sirtuins and chromatin,^{1,2} there are still many unaddressed issues as was clearly illustrated in the meeting. For instance, we still do not know all the targets of these enzymes, their chromatin location, or whether there is redundancy, antagonism or interplay between the different nuclear sirtuins as has been suggested. The presentations at the meeting helped to clarify that, although much effort is being invested in SIRT1 research, the field is expanding to incorporate the chromatin function of SIRT6 and SIRT7, and determine their contribution to the global sirtuin function.

One of the key unaddressed questions in the study of sirtuins is what are the targets through which they exert their function. In the context of chromatin, it has been clearly established that these enzymes not only deacetylate histones, but also other proteins, including transcription factors, histone methyltransferases, and histone acetyltransferases (see Fig. 1). However, our knowledge about the nature of these substrates remains incomplete. Therefore, the establishment of systematic screening assays to identify candidates seems to be a logical step forward. In this context, two different groups presented their current strategies. First, John Denu (UW Madison, WI, USA) described a new screening strategy based on a synthetic trifluoroacetyl-peptide library that should allow prediction of endogenous substrates for the different sirtuins. His

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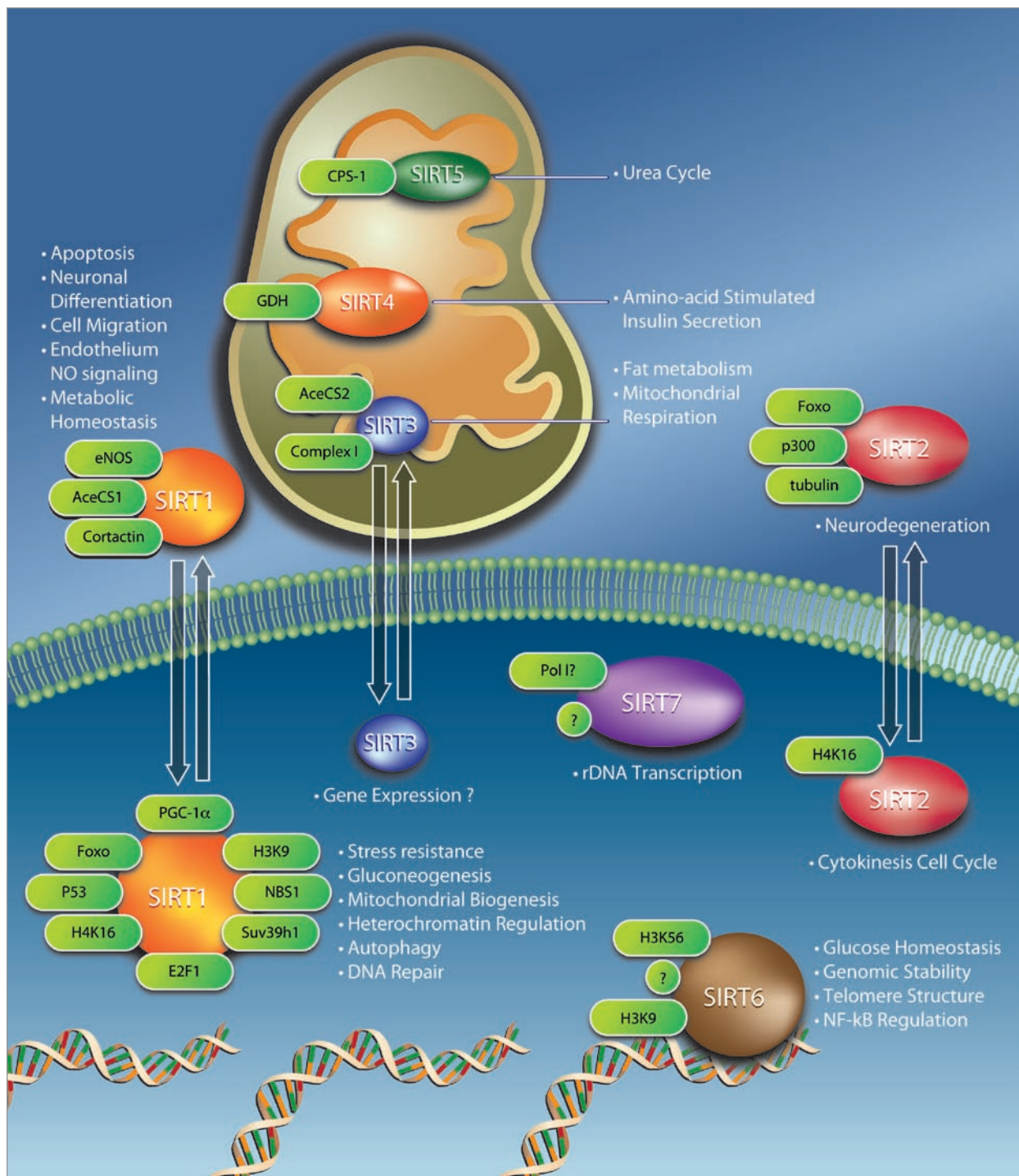


Figure 1. General overview of Sirtuin function. The distribution, targets (in green boxes) and representative functions (marked by dots) of the seven mammalian sirtuins are indicated. In the case of SIRT1, only a representative number of reported targets have been included due to space limitations of the figure. See Text for details.

group is currently validating several targets for SIRT3, and likely be able to successfully use this approach to identify novel substrates for the other sirtuins as well. Ed Seto (Moffitt Cancer Center, FL, USA) presented a novel method to identify

SIRT1 substrates based in Stable Isotope Labeling with Amino acids in Cell culture (SILAC) and anti-lysine antibodies. Many of the substrates obtained by the Seto lab are chromatin-related factors. Among them is the DNA methyltransferase

Dnmt1, which was previously shown to interact with SIRT1.³ Defects of DNMT1 has been associated with important nuclear and nucleolar aberrations^{3,4} and, in this setting, sirtuins might also be central to the epigenetic care-taking of the nucleus.⁵ The

Seto group was able to demonstrate that Dnmt1 is acetylated and, in turn, that it can be deacetylated completely by SIRT1. Although these intriguing findings need further study, they open many interesting questions regarding the interplay between sirtuins, DNA methylation and epigenetic silencing and suggest a closer relationship between sirtuins and DNA methylation than previously anticipated. Additional evidence linking SIRT1 with DNA methylation came from Ingrid Grummt (GCRC, Heidelberg, Germany), who provided new data regarding the role of SIRT1 in the regulation of rDNA silencing. SIRT1 deacetylates TIP5, the major subunit of NoRC, a SNF2 h-containing chromatin remodelling complex that silences a fraction of rRNA genes by establishing heterochromatic features at their promoters. SIRT1-dependent deacetylation of TIP5 increases binding of NoRC to pRNA, a noncoding RNA that is complementary to the rDNA promoter and is required for NoRC function.⁶ Knock-down of SIRT1 impaired NoRC-dependent heterochromatin formation, DNA methylation and silencing,⁷ highlighting the role of SIRT1 in epigenetic control mechanisms.

Another interesting contribution regarding the role of SIRT1 in heterochromatin formation came from Alejandro Vaquero (IDIBELL, Barcelona, Spain), who presented new data demonstrating a direct role for SIRT1 in the global organization of chromatin in the nucleus. In addition to a role in the formation of facultative heterochromatin through among other things a functional interaction with the H3K9me3 methyltransferase Suv39h1,^{8,9} SIRT1 seems to be involved in the formation of constitutive heterochromatin, also through a Suv39h1-dependent mechanism. SIRT1 regulates Suv39h1 in constitutive pericentromeric heterochromatin. The functional consequences of this novel mechanism seem to be very important since stress conditions that upregulate SIRT1 levels, such as calorie restriction, also upregulate Suv39h1 in a SIRT1-dependent manner. This actually provides the first evidence of a direct link between stress signalling and the chromatin organization keystone Suv39h1,¹⁰ and maybe one of the signalling ways to control genome stability and integrity.

Regarding the other nuclear sirtuins, SIRT6 and SIRT7, the meeting provided new evidence about previously unaddressed issues such as the genomic localization of these proteins. Katrin Chua (Stanford U., CA, USA) presented the first characterization of the chromatin regions bound by SIRT6 and SIRT7 using a ChIP-sequencing approach. The pattern of SIRT6 occupancy suggests that this protein regulates genes involved in chromatin architecture, transcription, and carbohydrate and RNA metabolism, among other functional gene categories. Surprisingly, SIRT7 was found at a variety of genes outside the nucleolus, and further data from the Chua lab suggests that SIRT7 has a repressive effect at these genes, in contrast to its active role in rDNA gene expression in the nucleolus through activation of RNA-polymerase I.¹¹ Together, these data provide an interesting view of the genome-wide distribution of SIRT6 and SIRT7 at chromatin, focusing in this case on a single cell type. The wealth of information obtained from this approach suggests that a highly promising avenue of investigation for future studies will be to take a similar approach to define the genome-wide distribution of SIRT6 and SIRT7 in other cell types, and physiologic contexts.

Another interesting issue raised in the meeting is whether the different chromatin-located Sirtuins might show a functional interplay. The most obvious case of such an interaction involves SIRT1 and SIRT7 since both have been found to localize to the nucleolus and regulate rDNA gene expression but in opposite ways: while SIRT1 promotes silencing,^{3,12} as mentioned above, SIRT7 is involved in the active transcription of these genes through RNA-polymerase I. Supporting an active interplay between both proteins, Eva Bober (MPI Bad Nauheim, Germany) provided novel results demonstrating that SIRT1 interacts with SIRT7 and that overexpression of SIRT1 excludes SIRT7 from the nucleolus. These findings suggest that Sirtuins regulate each other's activity thereby fine-tuning gene expression under conditions of stress.

Insight into the mammalian sirtuins has benefited extensively from studies of sirtuins in model organisms. Monika Jedrusik-Bode (MPI Göttingen, Germany)

provided new evidence regarding the characterization of the Sirtuin homologs in *C. elegans*. *C. elegans* contains four Sirtuins, Sir-2.1 to 2.4, of which only Sir-2.1, the ortholog of SIRT1, has been partially characterized. Sir-2.1, a histone deacetylase with preference for H3K9, associates with the telomeres of *C. elegans* and has synthetic effects with the linker histone subtype, HIS-24, on brood size, embryogenesis and fertility.¹³

Jedrusik-Bode also presented data regarding the other three sirtuins. Sir-2.4, the ortholog of SIRT6, is essential for life and localizes to chromatin but whether it regulates a subset of genes or has a general role is still unknown. The other two sirtuins, Sir-2.2 and Sir-2.3, the orthologs of SIRT4, do not localize to the nucleus and the expression pattern of both seems to overlap, with high expression of both factors in the pharynx and body wall muscles. Loss of both Sir-2.2 and Sir-2.3 results in a slightly “dumpy” phenotype (worms are shorter and fatter than wild type), suggesting a role of both sirtuins in the maintenance of energy accumulation to levels suitable for the organism welfare. This first characterization of these novel family members indicates that in worms, sirtuins have already evolved to play multiple functions. It is likely that future studies in this powerful genetic model system will provide further insight into our understanding of its mammalian counterparts.

The current landscape suggests that there is an unexpected degree of complexity in the functional link of sirtuins to chromatin which is reflected by a wide variety of substrates, a very dynamic localization, and an active interplay between the different nuclear sirtuins. Based on these findings, it seems certain that inquiry into how different physiological stimuli and stressing conditions such as oxidative or genotoxic stress shape chromatin structure, expression and organization, will be fruitful areas of future research.

Emerging Role for the Different Sirtuins in Metabolic Homeostasis

A common theme among the different participants was the consensus that, regardless of sub-cellular localization or tissue of expression, all seven mammalian sirtuins

appear to have evolved to play critical roles in modulating metabolism. Although several talks were focused on SIRT1, the most studied mammalian homolog so far, it was refreshing to see that all others sirtuins are actively being investigated as well (see Fig. 1). From the data presented, it is likely they are playing as important roles as SIRT1 in metabolism. Eric Verdin (Gladstone Institutes and UCSF, CA, USA), the Keynote Speaker, presented data indicating that expression of the mitochondrial SIRT3 protein is enhanced during fasting. SIRT3 deacetylates several mitochondrial proteins during fasting, and the Verdin laboratory and others are actively investigating the relevance of this activity.^{14,15} In this context, the Verdin laboratory took advantage of metabolomics to demonstrate that mice lacking SIRT3 show a selective defect in fatty acid oxidation. They identified long chain acylcoenzyme A dehydrogenase (LCAD), an enzyme in the fatty acid oxidation pathway, as a unique target of SIRT3 (see below). In this context, work presented by Francesc Villarroya (U. Barcelona, Spain) indicates that SIRT3 is upregulated in brown-adipose tissue in neonates, where it plays a critical role in regulating thermogenesis. Overall, these studies suggest that SIRT3 will emerge as a key player in regulating fat metabolism.

Another sirtuin that is emerging as an important regulator of metabolism is SIRT6. Raul Mostoslavsky (MGH-Harvard, USA) presented new results indicating a prominent role for SIRT6 in glucose homeostasis. SIRT6 deficient mice succumb early in life to a fatal hypoglycemia,¹⁶ however the reason for such a phenotype remained unclear. His laboratory now showed that SIRT6, through its previously defined H3K9 deacetylase activity,¹⁷ functions to repress multiple glycolytic genes, in this way promoting mitochondrial respiration when conditions of nutrient availability are propitious. Lack of SIRT6 activates expression of these glycolytic genes, causing a switch towards lactate production, with increased glucose uptake and concomitant inhibition of pyruvate entrance into the Krebs cycle in the mitochondria.¹⁸ They further demonstrated that this phenotype was dependent on the transcription factor Hif1 α , a known modulator

of nutrient- and oxygen-stress responses.¹⁸ Therefore, it appears that SIRT6 plays a critical role in modulating energy utilization in cells, depending on nutrient and oxygen conditions. Supporting these studies, Haim Cohen (U. Bar-Ilan, Israel) showed that transgenic mice overexpressing SIRT6 tolerate high-fat diet better than wild-type controls. These animals exhibit reduced accumulation of visceral fat, enhanced glucose tolerance and increased glucose-stimulated insulin secretion. Gene-expression analysis demonstrated that several genes associated with lipid storage were downregulated in adipose tissue of these animals.¹⁹ Whether this is a direct effect, or an adaptation to the effect of SIRT6 on glucose utilization remains unclear. Regardless, these results raise the exciting possibility that modulators of SIRT6 activity could be of therapeutic benefit in glucose-related diseases, such as diabetes.

Although the main substrate identified for SIRT6 is H3K9, recent studies have shown that SIRT6 functions as a histone H3K56 deacetylase as well (Fig. 1).^{17,20} Therefore, it is likely that this protein will play a broader role than previously thought. In this context, genome wide studies of SIRT6 promoter occupancy and conditional gene-targeting in specific tissues will be instrumental to fully understand the functions of this protein. Such studies are ongoing in the laboratories of Fred Alt (Children's Hospital, Harvard, Boston, USA), Katrin Chua and Mostoslavsky, so we should expect further progress on this sirtuin member in the near future.

Extensive biochemical characterization of SIRT1 suggests that SIRT1 may be the most multifaceted member of the sirtuin family, acting both as a chromatin histone deacetylase as well as a deacetylase of several non-histone proteins. However, for the long sought-after lifespan effect of this protein, *in vivo* studies are required, and Daniel Herranz (Serrano's lab, CNIO, Spain), presented new studies in this regard. Using a transgenic mouse overexpressing SIRT1, they showed that extra SIRT1 levels protected these mice against metabolic damage produced by high-fat diet.²¹ Both Pere Puigserver (Dana-Farber Cancer Institute, Boston, USA) and Johan Auwerx (Ecole Polytechnique Federal

Lausanne, Switzerland) reinforced their previous studies demonstrating that the main metabolic roles for SIRT1 (increased gluconeogenesis in liver and improved mitochondrial activity in muscle and adipose tissue) are dependent on SIRT1 deacetylation and activation of PGC-1 α . Auwerx and colleagues further hypothesized that if increased SIRT1 deacetylation of PGC1 α was beneficial under conditions of nutrient stress, then inhibiting the acetyl-transferase responsible for PGC-1 α acetylation should provoke a similar phenotype. To address this point, they generated mice deficient for the transcriptional co-activator SRC-3, the protein that controls expression of the acetyl-transferase GCN5. In support of their original hypothesis, these mice are lean and protected against diabetes and obesity when exposed to a high-fat diet, a phenotype dependent on decreased acetylation and increased activity of PGC-1 α .²²

The confluence of data pointing important metabolic functions for the sirtuins has motivated multiple laboratories to develop chemical modulators for these proteins. These compounds will provide valuable tools for both probing the biological function of these proteins as well as for testing the potential therapeutic benefits of modulating sirtuins under conditions of metabolic imbalance.

Sirtuins and Cancer

SIRT1 and SIRT2 functions are frequently altered in cancer cells (reviewed in ref. 23) and a diminished monoacetylated lysine 16 of histone H4, which is targeted by sirtuins, is a common hallmark of human tumors.²⁴ For these reason sirtuins are starting to be considered as targets for antitumorigenic therapy. Although in specific contexts SIRT1 appears to protect against tumorigenesis,²⁵⁻²⁸ several studies support an oncogenic role of sirtuins in cellular transformation. In this regard, Wei Gu (Columbia University, New York) provided further evidence of an oncogenic role of SIRT1 by showing that the tumor suppressor gene DBC1 (deleted in breast cancer 1) promotes p53-mediated apoptosis through specific inhibition of SIRT1. In addition, Katrin Chua (Stanford) presented data suggesting that SIRT7 is an

additional sirtuin that modulates oncogenic transformation and tumorigenesis.

The suggestive data supporting roles for sirtuins in cancer, has led to efforts to pharmacologically target these proteins. To date, the majority of such research has focused in the development of compounds that block and diminish the activity of sirtuins. The first-known Sirtuin inhibitors can be classified into two groups: the substances that inhibit NAD⁺ dependent reactions in general, such as nicotinamide,^{29,30} and Sirtuin specific inhibitors, such as splitomicin,³¹ sirtinol,³² cambinol,³³ dihydrocoumarin³⁴ and some indoles.³⁵ Notably, these compounds share the common feature of having antitumour properties. However, their effects are generally dependent on the tumour type and stress conditions, their mechanisms of action are varied or are still unclear, and their effect on normal cells has not been studied thoroughly. During the meeting, Manel Esteller (Cancer Epigenetics and Biology Program, Barcelona) described the synthesis and mechanism of action of Salermide (N-{3-[(2-hydroxy-naphthalen-1-ylmethylene)-amino]-phenyl}-2-phenyl-propionamide), a new molecule with a potent inhibitory effect on SIRT1 and SIRT2.³⁶ He also showed the potent antitumoral effect of newly developed sirtuin inhibitors in a mouse model of radio-induced T-lymphomas, previously used for the development of DNA methyltransferase inhibitors.³⁷ Antonio Bedalov (Fred Hutchinson Cancer Research Center, Seattle, USA) also demonstrated that B-lymphomas are also sensitive to the sirtuin inhibitor Cambinol, which induced acetylation of both p53 and BCL6. Sonia Lain (Karolinska Institute, Stockholm, Sweden) introduced the tenovin-6 compound,³⁸ initially discovered using a 30,000 compounds-small molecule library to identify p53 activators, that is able to inhibit the protein-deacetylase activities of SIRT1 and SIRT2. All researchers agreed that sirtuin inhibitors have a great potential as anticancer drugs and stressed the necessity to understand the different molecular specificities of each compound to design a roadmap for future trials in cancer patients.

Conclusions

Although they were discovered only ten years ago, the study of mammalian sirtuins has become one of the most promising areas of biomedicine, given the newly established link between this family of proteins and many important human pathologies. These proteins appear to both sense oxidative stress and to promote a cellular response to these conditions. This response, which includes protecting the genome and optimizing the energy resources, is based on a dual role of Sirtuins as regulators of both chromatin, where they are involved in chromatin expression, structure and organization, and mitochondria, where they support a tight regulation of cellular energy.

Despite the fact that many aspects of Sirtuin Biology remain poorly understood, this meeting contributed remarkably to highlight how much we have learned so far, and marked the paths through which this new field should tread in the near future.

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