Regulation of cellular homoeostasis by reversible lysine acetylation

Iain Scott

Center for Molecular Medicine, National Heart, Lung, and Blood Institute, National Institutes of Health, Room 5-3216, Building 10-CRC, 9000 Rockville Pike, Bethesda, MD 20892, U.S.A.

Abstract

Acetylation, through the post-transcriptional modification of histones, is a well-established regulator of gene transcription. More recent research has also identified an important role for acetylation in the regulation of non-histone proteins, both inside and outside the nucleus. As a fast (and reversible) post-translational process, acetylation allows cells to rapidly alter the function of existing proteins, making it ideally suited to biological programmes that require an immediate response to changing conditions. Using metabolic programmes as an example, the present chapter looks at how reversible acetylation can be used to regulate important enzymes in an ever-changing cellular environment.

Introduction

Organisms exist in a constant state of flux and, therefore, require the ability to adapt to changes in their environment. At the cellular level, these environmental changes (whether they are metabolic, nutritional, protective etc.) can generally be dealt with by altering the amount and activity of specific regulatory proteins.
When environmental changes occur slowly, the cell can respond by producing greater amounts of any protein it needs to maintain physiological homoeostasis. However, when rapid changes occur that require an immediate response, the cell cannot afford to wait for the transcription/translation machinery to meet the demand. In these cases, cells often have the required proteins and mechanisms already in place to take care of any problems. Although it is important for these types of proteins to exist at all times, it is equally important that the cell can switch them on and off when required. For example, leaving many proteins continually activated could lead to a high energetic burden for the cell, especially if the activated protein requires biochemical energy (ATP, GTP etc.) to function. As such, many fast-acting biochemical mechanisms have evolved to act as regulators of protein activity, and these include the PTM (post-translation modification) of proteins.

PTMs usually involve the addition of small chemicals or proteins to enzymes, which act to increase or diminish their activity. One of the most widely studied PTMs is lysine acetylation, which involves the addition of an acetyl group to the ε-amino group of lysine residues by a lysine acetyltransferase. This process is reversible, and a second group of enzymes, deacetylases, can remove this acetyl group and return the substrate protein to its former state. Acetylation, as a PTM, was first discovered in the 1960s in studies investigating histones [1]. During gene expression or DNA replication, histones can become acetylated. This changes the charge of the protein and reduces its affinity for DNA, allowing genes to become more accessible to polymerases [2]. More recent work has established that lysine acetylation is not restricted to histones, and is in fact present in a huge number of protein groups [3–6]. These acetylation/deacetylation events have been shown to be involved in many processes, regulating mechanisms as disparate as cytoskeletal dynamics, fat metabolism and protein localization.

Using mainly glucose homoeostatic pathways as an example, this chapter will introduce the main mechanisms involved in regulating the acetylation status of non-histone proteins, before focusing on how sirtuin deacetylases control multiple aspects of mammalian metabolism.

**Controlling the acetylation status of proteins in the nucleus and cytoplasm**

As stated above, lysine acetylation is a reversible PTM and is carried out by opposing sets of enzymes called acetyltransferases (addition of an acetyl group) and deacetylases (removal of an acetyl group) (please see Chapter 1 for a detailed review). Many acetyltransferases and deacetylases are found in discrete cellular locations (e.g. restricted to the nucleus); however, some have the ability to shuttle back and forth between compartments (e.g. from the nucleus to the cytoplasm) under different physiological conditions. The acetyl groups added by acetyltransferases come from the coenzyme acetyl-CoA,
and are added to the ε-amino group of protein lysine residues. As such, the acetylation status of any substrate protein is the result of a combination of three basic factors: (i) the current activity of its specific acetyltransferase(s), (ii) the current activity of its deacetylase(s), and (iii) the availability of acetyl-CoA as an acetylation substrate. Adding another layer of complexity is the fact that proteins may contain multiple lysine residues, and the acetylation status of different residues may be regulated by different acetyltransferases and deacetylases. The differential acetylation of lysine residues on the same protein may then lead to different biological outcomes (e.g. deacetylation of one residue may activate a protein, whereas the same process at a different residue may inactivate it). Finally, there may be cross-talk between different types of lysine PTM (ubiquitination, SUMOylation, methylation etc.), all of which occur at the same lysine residue ε-amino group. As such, the addition of another type of lysine PTM may block the ability of a protein to become acetylated, thereby attenuating regulation using this process. Clearly, the control of protein function by acetylation requires the co-ordination of multiple cellular regulation systems.

Acetyltransferases
There are three major acetyltransferase [also known as HATs (histone acetyltransferases) or, more accurately, KATs (lysine acetyltransferases)] families, and member proteins from each group have been implicated in the control of cellular homoeostasis (reviewed in [7]). The GNAT [GCN5 (general control of amino acid synthesis 5)-related N-acetyltransferase] family is very large and contains numerous proteins, such as PCAF (p300 (E1A-associated protein of 300 kDa)/CBP [CREB (cAMP-response-element-binding protein)-binding protein]-associated factor], ELP3 (elongation protein 3) and GCN5, which is the archetypal member of the group and the first KAT to have been discovered [8]. The second family, MYST acetyltransferases, contains TIP60 [Tat (transactivator of transcription)-interactive protein 60 kDa], MOZ (monocyctic leukaemic zinc-finger protein), HBO1 (histone acetyltransferase binding to Orc1), MORF (MOZ-related factor) and MOF (male absent on first). These proteins have a wide range of acetylation substrates, and regulate pathways both in the nucleus (transcription, DNA repair etc.) and the cytoplasm (e.g. gluconeogenesis). Finally, the third group of KATs contains two homologous members, p300 and CBP, which are well characterized as transcriptional activators. Acetyltransferases often form large multimeric complexes with accessory proteins which may facilitate, or be required, for their activity [9]. Searches for small molecule inhibitors of KAT function have identified several synthetic and natural chemicals, such as curcumin and garcinol, which have potential pharmaceutical applications [7]. Given the number of KATs characterized in the human genome (~20), and the number of known acetylated proteins (~1750), it would appear that these enzymes have a wide substrate specificity.
Deacetylases

Like KATs, deacetylase proteins [also known as HDACs (histone deacetylases) or KDACs (lysine deacetylases)] are grouped into families. The class I (HDAC1–HDAC3 and HDAC8) and class IV (HDAC11) KDACs are predominantly localized in the nucleus, whereas the class II KDACs (HDAC4–HDAC7, HDAC9 and HDAC10) can shuttle between the nucleus and the cytoplasm [10]. Class III KDACs, which are NAD$^+$-dependent deacetylases, are described below. Class I, II and IV KDACs are zinc-dependent enzymes, which have a conserved catalytic domain consisting of two histidine residues and a zinc ion [10]. These enzymes, whose activity can be blocked using TSA (trichostatin A), deacetylate a wide range of nuclear and cytosolic proteins including transcription factors [such as p53 and NF-$\kappa$B (nuclear factor $\kappa$B)] and cytoskeletal proteins (such as $\alpha$-tubulin).

Sirtuins: deacetylases that regulate numerous metabolic pathways

One of the most interesting groups of deacetylase enzymes, in terms of cellular homeostasis, is the class III KDACs, also known as the sirtuins. Mammalian sirtuin proteins are named after Sir2 (silent information regulator 2), a gene that was identified as controlling lifespan in yeast [11]. In humans there are seven members of the sirtuin family (SIRT1–7), and the different proteins can be found in discrete cellular locations (e.g. SIRT1 in the nucleus and SIRT3 in mitochondria). The majority of the proteins exhibit KDAC activity, and appear to function in numerous cellular pathways, removing acetyl groups in order to increase or decrease the activity of their substrates.

In terms of metabolism, sirtuin proteins are intricately linked to the energy status of the cell through the coenzyme NAD$^+$. NAD$^+$ (and its reduced form NADH) acts as an electron transfer protein in metabolic redox reactions, with the ratio between the electron-accepting and -donating forms regulating the activity of many enzyme pathways. Sirtuin proteins are linked to this energetic control by two complementary mechanisms. First, sirtuins are NAD$^+$-dependent enzymes, and their activity is enhanced when cellular NAD$^+$ levels are increased. Secondly, one of the by-products of the NAD$^+$-dependent sirtuin deacetylation reaction is NAM (nicotinamide), which acts as an inhibitor of sirtuins [12]. In effect, the enzymatic activity of these proteins contains an in-built regulation system to prevent excessive deacetylation of target proteins. This negative-feedback control remains in place until the energetic status of the cell changes, and the renewed requirement for free NAD$^+$ can be met, in part, by the conversion of NAM into NAD$^+$ through metabolic salvage pathways (Figure 1).

The regulation of metabolism by sirtuin proteins has made them a prime target for research into related human diseases. Several major pathological conditions (including cancer, Type 2 diabetes and neurological disorders) are caused or
characterized by defects in metabolic control (reviewed in [13]). The ability of sirtuin proteins (particularly SIRT1 and SIRT3) to mediate mitochondrial biology and bioenergetics is especially important. Mitochondria are the primary site of damaging ROS (reactive oxygen species) production, and the deacetylation function of sirtuin proteins is crucial in the regulation of antioxidant genes which ameliorate ROS damage [14]. ROS damage has been linked to loss of mitochondrial function, which is a major factor in the development of metabolic diseases (such as diabetes) and aging-related pathologies (such as Alzheimer’s disease and Parkinson's disease) [13]. The role of sirtuin proteins in human disease is currently expanding rapidly, and will no doubt be an area of great importance for some time to come.

Although our understanding of the regulation of sirtuin enzyme activity has greatly increased, it is the downstream effects of sirtuin function that have received the most focus. Therefore the role of three major sirtuin deacetylase proteins, SIRT1, SIRT2 and SIRT6, in the regulation of several cellular homoeostatic pathways is discussed below.

**SIRT1**

SIRT1 is the archetypal member of the mammalian sirtuin family and is the major nuclear deacetylase in this group. One major area of SIRT1 research has been its role in CR (caloric restriction), which has long been known to increase lifespan in a variety of organisms. CR was first shown to have an effect on sirtuin
activity in yeast, where the reduction in calories caused an increase in available NAD\(^+\), which is required for sirtuin function \[11\]. The ability of SIRT1 homologues to increase lifespan in various organisms led to the search for novel chemical activators. One of the best known is resveratrol, an antioxidant found in red wine. Resveratrol improves the enzyme activity of SIRT1 by increasing its ability to bind and deacetylate substrates \[15\].

Given the NAD\(^+\) requirement in sirtuin function, systems which regulate the intracellular NAD\(^+\)/NADH ratio have a powerful role in regulating SIRT1. AMPK (AMP-activated protein kinase), a key energy homoeostasis enzyme, helps to regulate this balance. One of the first studies to link AMPK function and SIRT1 activity looked at glucose metabolism. This found that reduced levels of glucose led to the activation of AMPK, which induced the NAD\(^+\) salvage pathway to create more NAD\(^+\), which activated SIRT1 \[16\]. These results were further extended by studies demonstrating that AMPK, acting as a cellular fuel sensor, could increase the transcriptional activity of proteins regulated by SIRT1 \[17\]. The depletion of cellular energy leads to the activation of AMPK, which stimulates SIRT1 in the manner described above. SIRT1 then deacetylates, and activates, several transcription regulators, such as FOXO (forkhead box O) proteins and PGC-1\(\alpha\) (peroxisome-proliferator-activated receptor \(\gamma\) co-activator 1\(\alpha\)) \[18\].

PGC-1\(\alpha\) is a major transcriptional co-activator, and is involved in cellular metabolism and mitochondrial biogenesis (the formation of new mitochondria). It has been demonstrated that, in the liver, SIRT1 is activated in response to fasting, which leads to the deacetylation of PGC-1\(\alpha\) \[19\]. Deacetylated PGC-1\(\alpha\) can then bind to the promoter regions of fasting-response genes and regulate how they function. In this case, the lowered availability of nutrients in the cell led to PGC-1\(\alpha\) switching on genes that produce more glucose (through gluconeogenesis; see below) and switching off genes that consume glucose, such as those involved in glycolysis \[19\]. When nutrient conditions return to normal, acetylation is again used to modulate PGC-1\(\alpha\) function. To counter the activity of SIRT1, a nuclear-localized KAT called GCN5 acetylates PGC-1\(\alpha\) at specific residues. This causes the transcriptional co-activator to detach from the promoter regions of its target genes and form non-functional foci in the nucleus \[20\]. To summarize, reversible acetylation, involving counteracting KATs and KDACs, is central to the transcriptional control of glucose homoeostasis.

**SIRT2**

SIRT2 is a unique member of the sirtuin family, in that many of its functions occur in the cytoplasm. Although much less studied than SIRT1, it is becoming increasingly apparent that SIRT2 is involved in the regulation of numerous pathways. Much of the early research into SIRT2 focused on its role in controlling aspects of cellular architecture, particularly the cytoskeleton. SIRT2 was shown to interact with, and deacetylate, tubulin, a key protein in
microtubules. Changes in the acetylation status of tubulin has an impact on the stability of microtubules, and therefore the deacetylation of tubulin by SIRT2 may be involved in regulating changes to cytoskeletal architecture (reviewed in [21]). These changes also linked SIRT2 to the cell cycle, as major changes in the cytoskeleton are required during mitosis. It has been shown that an increase in SIRT2 protein levels occurs at the G2/M transition of the cell cycle, and that cells overexpressing a deacetylase-null mutant copy of SIRT2 were slowed at this stage [22].

More recent research has shown that SIRT2, and the opposing KAT p300, regulate a major energetic homoeostatic mechanism. When organisms face low-nutrient conditions (e.g. fasting, scarcity of food/prey etc.), several metabolic pathways can be activated which increase the availability of nutrients to cells. To prevent hypoglycaemia, mammals can activate two main pathways that lead to increased cellular glucose concentrations: glycogenolysis (the breakdown of stored glycogen) or gluconeogenesis (the de novo formation of glucose from non-carbohydrate sources such as lactate or amino acids). The first irreversible rate-limiting step of gluconeogenesis is the conversion of oxaloacetate into phosphoenolpyruvate, which is carried out by the enzyme PEPCK1 (phosphoenolpyruvate carboxykinase). A proteomics screen of metabolic pathways in 2010 identified this enzyme as an acetylation substrate [6], indicating that the process may be regulated by this PTM. Although this turned out to be true, this regulation was not as simple as perhaps first imagined.

PEPCK1 is activated under low-glucose conditions, which drives gluconeogenesis in the liver (and to a lesser extent, the kidneys), leading to an increase in available glucose. At the least-complex level, the acetylation of PEPCK1 would theoretically act as a molecular switch, with the addition of an acetyl group switching the protein on or off (with deacetylation acting in the opposite direction). However, the regulation of PEPCK1 has evolved in a more complex manner, and involves the interaction of two lysine PTMs. When glucose levels are low, SIRT2 is transcriptionally activated, leading to increased protein abundance and deacetylation activity. SIRT2 then deacetylates PEPCK1, which leads to a stabilization of this protein and an increase in gluconeogenesis [23]. Under the opposite nutrient conditions the levels of SIRT2 are diminished and the abundance and activity of the counteracting KAT (p300) are increased. p300 acetylates PEPCK1 under these high-glucose conditions, which, using our simple theory, would be enough to block PEPCK1 function. However, this regulation goes a step further, as the acetylation of PEPCK1 by p300 causes it to be removed altogether. When PEPCK1 is acetylated, it marks it for ubiquitination by the Ub (ubiquitin) E3 ligase UBR5. This ubiquitinated PEPCK1 is then targeted for degradation by the proteasome, completely inhibiting gluconeogenic activity [23]. Therefore reversible acetylation (by p300 and SIRT2) not only regulates this major pathway, it provides a mechanism for two very different lysine PTMs to work together.
SIRT6
SIRT6 is a nuclear-localized deacetylase, which controls many pathways by regulating (or co-regulating) processes related to transcription and DNA interactions. SIRT6 is thought to display a weaker deacetylase activity (approximately 1000-fold less) than some other sirtuins, but can still catalyse the reaction as required [24]. So far the majority of research on SIRT6 has focused on its true HDAC activity, and it has been shown to remove the acetyl groups from lysine residues of histone H3 at Lys9 (H3K9) and Lys56 (H3K56) (reviewed in [25]). This function has linked SIRT6 to DNA repair [for example at DSBs (double-strand breaks) caused by oxidative damage] and the maintenance of telomeres [25]. With regard to cellular homeostasis, SIRT6 has been found to have an important role in the transcriptional response to changes in glucose metabolism.

Many alterations in cellular homeostasis are regulated at the transcriptional level, and one of the main transcription factors involved is HIF1α (hypoxia-inducible factor 1α). This protein, originally identified as a factor mediating responses to cellular hypoxia, has been implicated in several other capacities. To be activated, HIF1α is acetylated by the KAT p300, and this leads to the initiation of target gene transcription (reviewed in [26]). Under normal glucose concentrations, SIRT6 interacts with HIF1α and deacetylates it, preventing the transcription factor from activating genes. This allows normal cellular energetic pathways (such as increased mitochondrial oxidative phosphorylation and decreased glycolysis) to take place [27]. However, when the energy balance of the cell shifts to low-glucose conditions, SIRT6 is inactivated and HIF1α becomes acetylated (most probably by p300) and its transcriptional activity is enhanced [27]. Further research has shown that SIRT6 directly binds to, and deacetylates, the promoter regions of several major glycolytic genes (such as lactate dehydrogenase and pyruvate dehydrogenase kinase 1), indicating that SIRT6 may be a master regulator of cellular glucose metabolism at the transcriptional level [26].

Conclusions
Despite being a relatively young field of research, our knowledge of how proteins are regulated by lysine acetylation has grown at a prodigious rate. In the space of approximately 15 years, we have identified suites of acetyltransferases and deacetylases, established their enzymatic functions and catalogued some of their substrates; however, it is clear from recent proteomic studies that we have so far only scratched the surface of acetylation. Hundreds (if not thousands) of proteins in every compartment of the cell have now been identified as lysine acetylation substrates, and there is much still to discover about what all of these modifications mean in terms of cellular function. For each acetylated protein, we have several questions to ask. What enzyme adds or removes the acetyl group? How is it regulated? How does it change the function of the protein? How does acetylation interact with other lysine PTMs?
When it comes to cellular metabolism and physiology, lysine acetylation takes on an even deeper significance. The base unit of acetylation, the acetyl group of acetyl-CoA, is a breakdown product of fuel metabolism. The sirtuins, one of the most metabolically relevant deacetylase groups, are driven by fundamental biochemical reactions that are closely linked to cellular energetic states. Almost every metabolic pathway studied contains enzymes that are modified, and perhaps regulated, by acetylation. It is clear, therefore, that acetylation and metabolism are intricately linked. As such, future work will be required to delineate how much feedback regulation there is between global cellular homoeostasis and global protein acetylation.

Summary

- Lysine acetylation is a wide-spread, rapid and reversible PTM.
- The counteracting activity of acetyltransferases and deacetylases regulates the acetylation status, and thus activity, of a vast number of proteins.
- Cellular programmes that need to react quickly to changing conditions, such as metabolic pathways, are prime targets of regulation by lysine acetylation.
- The sirtuin family of lysine deacetylases both regulate and are regulated by cellular metabolic and physiological conditions.

The work of the author is supported by the intramural research programme of the National Heart, Lung and Blood Institute (NHLBI) at the National Institutes of Health. The author is deeply indebted to his mentor, Michael Sack, and wishes to thank him for his help and support.

References


© The Authors Journal compilation © 2012 Biochemical Society

© The Authors Journal compilation © 2012 Biochemical Society