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Defining the Orphan Functions of Lysine Acetyltransferases

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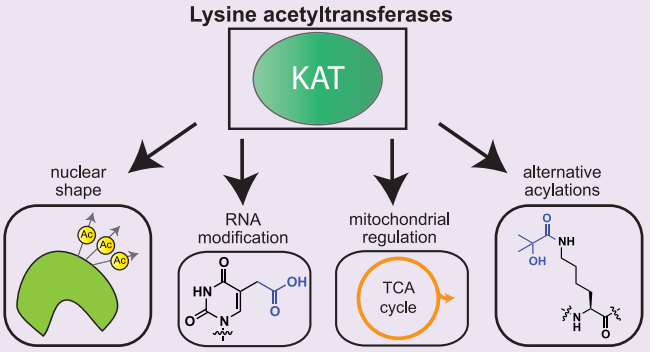
ABSTRACT: Long known for their role in histone acetylation, recent studies have demonstrated that lysine acetyltransferases also carry out distinct "orphan" functions. These activities impact a wide range of biological phenomena including metabolism, RNA modification, nuclear morphology, and mitochondrial function. Here, we review the discovery and characterization of orphan lysine acetyltransferase functions. In addition to highlighting the evidence and biological role for these functions in human disease, we discuss the part emerging chemical tools may play in investigating this versatile enzyme superfamily.



Acetylation is a macromolecular modification well-known for its role in histone modification and regulation of genomic function (Figure 1). The first enzyme catalyzing this process, the lysine acetyltransferase (KAT) Gcn5, was identified in 1995 from the protozoan Tetrahymena on the basis of its ability to transfer radiolabeled acetyl-CoA to a histone impregnated gel slice.[1] In the 20 years since this discovery, our understanding of the role of acetylation in biology has evolved rapidly.[2] The biomedical impact of this revolution has been similarly rapid and remarkable.[3] KAT function is antagonized by lysine deacetylase (KDAC) enzymes, and inhibitors of this process have been clinically approved for the treatment of T-cell lymphoma.[4,5] More recently, inhibitors of bromodomains—structural motifs that interact with the KAT-catalyzed acetyl-lysine modification—have also shown anti-tumor efficacy in preclinical studies.[6,7]

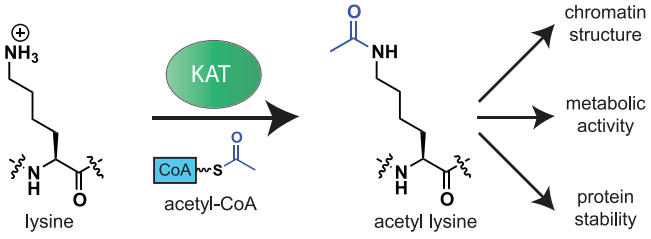


Figure 1. Regulation of protein activity by acetylation. Lysine acetyltransferase (KAT) enzymes catalyze nucleophilic attack of the electrophilic acetyl-CoA thioester by the ϵ-amine of protein lysine residues. This modification is recognized by bromodomains and removed by lysine deacetylase (KDAC) enzymes (not shown). Through these modificatons, lysine acetyltransferases can modulate diverse cellular phenomena including chromatin structure, enzyme activity, and protein stability.

The finding that therapeutic benefits can be derived from both targeting the proteins that remove acetylation (KDACs) and those that recognize it (bromodomains) exemplifies the complexity of acetylation biology. This complexity was compounded in 2009, when the advent of new immunoprecipitation methods and improved analytical tools led to the discovery that a large portion of the human proteome (now estimated at over 4000 sites) are subject to lysine acetylation (Figure 1).[8,9] One implication of these findings is that the cellular acetyltransferase program may be more diverse than previously recognized. However, while tremendous advances have been made in our understanding of cellular KAT function, these studies have focused heavily on a few main enzyme families: Gcn5/pCAF, p300/CBP, and MYST. These "canonical" KATs have been the subject of extensive characterization due to their essential roles in transcription, replication, and DNA repair, work summarized in several excellent articles.[10−14] In this review, our goal is to complement knowledge gained in the study of these core KAT families by shifting our focus to less well-known and emerging paradigms in KAT biology, specifically what we term "orphan" KAT functions. Many of these functions do not involve modification of histones, are not carried out in the nucleus, and mediate biological functions distinct from transcription. Specifically, we focus on (1) p300, a canonical KAT with the ability to install non-nuclear acetyl- and acyl-lysine modifications; (2) Elp3, a KAT involved in RNA modification; (3) Nat10 and (4) Gcn5L1, two recently characterized KATs that play roles in tubulin and mitochondrial acetylation, respectively; and (5) Acat1, a primary metabolic enzyme that recent evidence suggests moonlights as a mitochondrial KAT. By focusing on orphan KAT function through the prism of these five enzymes, we seek to emphasize findings that add to previously established concepts and highlight new directions of inquiry. Finally, we conclude with

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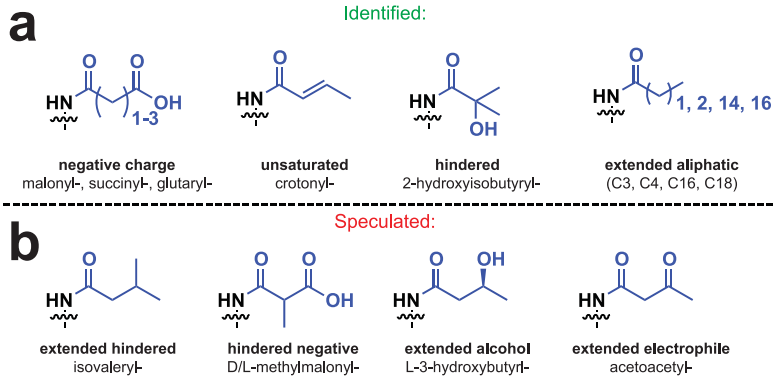

Figure 2. Lysine acylations derived from nonacetyl, metabolic acyl-CoA cofactors. (a) Lysine acylations that have been identified or analyzed in functional studies to date. Notably, each family of modification imbues the lysine side-chain with a unique physiochemical property. (b) Examples of lysine acylations that have not been identified but that may be hypothesized to exist based on the abundance of their cellular acyl-CoAs. Notably, each of these speculative modifications would imbue the lysine side-chain with novel physiochemical properties.

a summary of open questions and potential paths forward for the field.

A WELL-KNOWN KAT RESPONSIBLE FOR ORPHAN MODIFICATIONS: p300

The transcriptional coactivator p300 is a large (300 kDa) acetyltransferase enzyme found in all metazoans.[10] The p300 KAT domain shares 87% amino acid identity with another acetyltransferase, CBP, although the two proteins are functionally distinct. Despite being one of the first discovered and most well-characterized KAT enzymes,[15] recent literature suggests p300 also possesses some less well-understood and emerging functions. These derive from its ability to utilize a diverse range of substrates and acyl-CoA cofactors, which we term p300's "orphan" activities.

Focusing on acetylation, p300 has been shown to catalyze acetylation of nonhistone targets such as transcription factors, metabolic enzymes, kinases, other KATs, cytoskeletal components, and a multitude of other proteins (Figure 1). This is facilitated biochemically by p300's utilization of a relatively uncommon Theorell-Chance mechanism, in which the protein lysine substrate is proposed to bind rapidly to the preformed p300/acetyl-CoA complex, and undergo acyl transfer without formation of a stable ternary complex.[16,17] One example illustrating this radical substrate tolerance came during recombinant expression of the p300 domain in *E. coli*. In this study, Cole and co-workers found that p300 expression was greatly improved by coexpression of the yeast KDAC Sir2. The implication of this finding was that overexpressed human p300 has sufficient promiscuity to acetylate bacterial protein targets, and that it does so to a degree that promotes toxicity.[18] These findings suggest caution may be warranted when attributing the acetylation of specific substrates to the activity of p300 (or other canonical KATs) based on overexpression experiments alone.

In addition to diverse substrates, an emerging body of literature indicates that lysine residues are modified by diverse acyl-modifications, including propionyl-, butyryl-, malonyl-, succinyl-, crotonyl-, hydroxybutyrl-, 3-phosphoglyceryl-, palmitoyl-, and myristoyl (Figure 2a).[19−23] Indeed, while by no means a *fait accompli*, at this point it may be reasonable to speculate that most metabolic acyl-CoAs are capable of modifying lysine residues and that additional modifications will be discovered as analytic tools become more powerful (Figure 2b). A major challenge lies in understanding how and/or whether these marks are regulated. Notably, some KDACs have been found to remove alternative acyl-CoA modifications with vastly different rates (reviewed in this journal).[24] While the KATs governing this process are less well-understood, an early study demonstrated that p300 is able to utilize propionyl- and butyryl-CoA as histone acylation cofactors *in vitro*.[23] This property is shared by CBP, Gcn5, and pCAF.[25,26] The transfer of these longer acyl-chains occurs more slowly than acetylation.[25] Hang and co-workers exploited this property to identify direct p300 substrates by utilizing pentynoyl-CoA, an extended acyl-CoA bearing a latent affinity handle whose transfer to lysine substrates could be enriched and detected by LC-MS/MS.[27] In addition to propionylation (C3), butyrylation (C4), and pentynoylation (C5), a recent publication reported p300 is capable of transferring succinyl- (C4) and glutaryl-CoA (C5) acyl units to a histone H4 peptide substrate (Figure 2).[28] While this phenomenon will require kinetic characterization in order to understand its physiological relevance, it suggests p300 can also accommodate negatively charged acyl-CoA cofactors in a conformation that is compatible with the binding of protein substrates.

While such studies are in their early stages, an emerging hypothesis is that the cofactor promiscuity exhibited by p300 (and other KATs) may allow them to act as metabolic sensors, relaying signals about cellular acyl-CoA levels to histone or nonhistone proteins to mediate their activity.[29,30] Such a role would be consistent with recent studies demonstrating p300 acts as a metabolic sensor in suppressing autophagy under conditions in which nucleocytosolic acetyl-CoA levels are elevated.[31] Interestingly, biochemical characterization of p300 indicates the enzyme possesses a K_m for acetyl-CoA far below measured cellular acyl-CoA concentrations. This suggests p300 may be sensitive to subcellular fluctuations in local, rather than global, acetyl-CoA levels. Determining how KAT catalytic activity is affected by the availability and subcellular biosynthesis of alternative acyl-CoA cofactors will be an important step toward defining their functional connection to metabolism *in vivo*.

JACK OF ALL TRADES (PROTEIN, RNA, DNA?): Elp3

Elp3 was first identified as the catalytic KAT subunit of Elongator, a multiprotein complex that copurifies with transcriptionally engaged RNA Polymerase II (RNAPII) in

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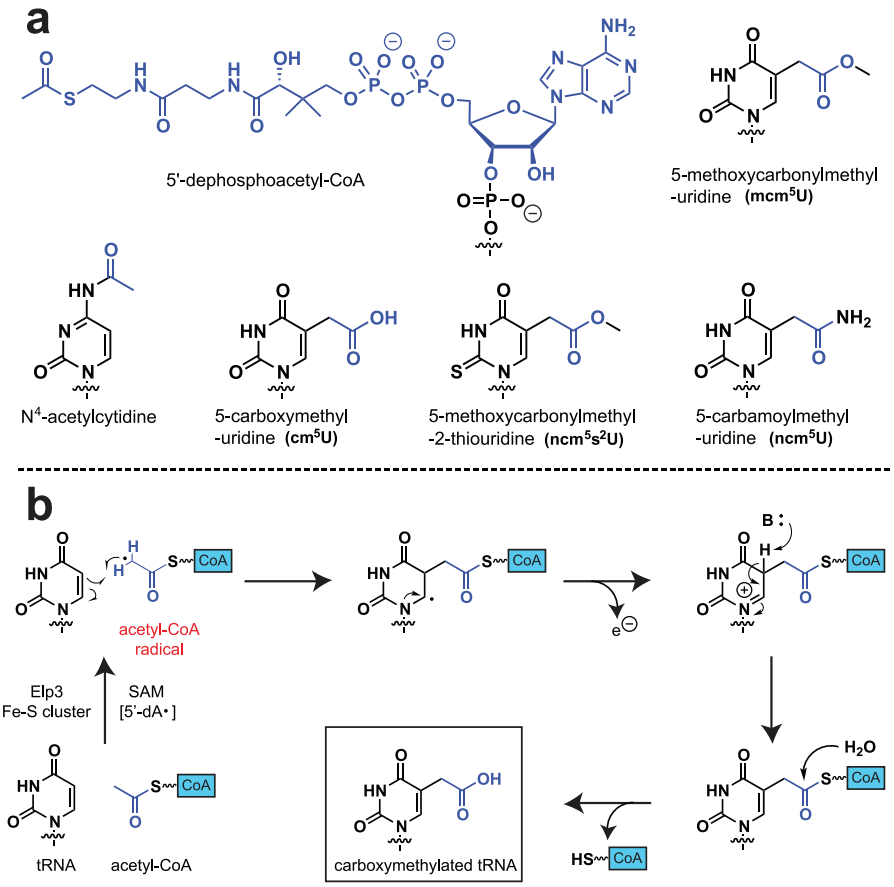

Figure 3. Acetyl-CoA dependent modifications of RNA. (a) Examples of acetyl-CoA derived modifications that have been identified in prokaryotic and eukaryotic organisms. The acetyl-CoA derived portion of each RNA modification is pictured in blue. (b) Mechanism of Elp3-catalyzed tRNA modification. Binding of S-adenosine methionine (SAM) and tRNA catalyzes formation of an adenosyl-radical by the Elp3 Fe−S cluster, which, in turn, abstracts a hydrogen from acetyl-CoA. The acetyl-CoA radical undergoes C−C bond formation with adenosine, followed by electron loss, and general base catalysis to form the acetyl-CoA-RNA adduct. Subsequent hydrolysis yields the carboxymethylated tRNA product.

yeast.[32] Subsequent studies demonstrated Elp3 is biochemically competent as a KAT, can facilitate acetyl-CoA-dependent RNAPII transcription through chromatin, and is required for a subset of H3 acetylation events in cells.[33−35] In addition to histones, Elp3 and its species-specific homologues have also been demonstrated to acetylate other proteins including tubulin,[36] the *Drosophila* neuronal protein Bruchpilot,[37] and the pentose phosphate enzyme glucose-6-phosphate dehydrogenase.[38]

While these findings mark Elp3 as a versatile and biologically relevant KAT, this enzyme's "orphan" designation arises from its ability to facilitate acetyl-CoA-based modification of a remarkably different class of macromolecule: RNA. Several acetyl-CoA-based modifications of RNA have been identified (Figure 3a). These include 5-carboxymethylation of uridine, found in the modified nucleosides cm^5U, mcm^5U, mcm^5s^2U, and ncm^5U (generally referred to here as xm^5U).[39] These modifications are implemented post-transcriptionally and can be found in over 25% of yeast tRNAs, where they help regulate mRNA decoding and translational fidelity. Elp3's involvement in RNA modification was identified through the study of a fission yeast mutant demonstrating defective suppressor tRNA function.[40] Genetic analyses mapped the mutation to the Elp3 locus, and it was found that an active Elp3 KAT domain was required for incorporation of xm^5U modifications into yeast tRNA. This finding helped explain the molecular basis for the observation that genetic ablation of Elp3 confers resistance to the killer yeast toxin zymocin, an xm^5U-specific RNase.[41] Furthermore, Bystrom and co-workers determined that many of the phenotypes associated with Elp3 loss in budding yeast, including temperature-sensitive growth, could be suppressed by overexpression of two tRNA genes.[42] Interestingly, overexpression of these two tRNAs also restores histone acetylation defects observed in Elp3-deficient yeast. This potentially indicates that RNA regulation is Elp3's primary function and that downstream effects (such as loss of specific histone acetylation events) represent indirect epi-phenomena.

While these studies provided strong evidence for the involvement of Elp3 in RNA modification, the mechanistic basis for this activity was only recently described. Elp3 is unique among known multidomain acetyltransferases in that it houses an iron−sulfur (Fe−S) cluster, frequently associated with radical SAM enzymes. Evidence for the participation of this domain in RNA carboxymethylation was provided by its high conservation, as well as studies showing Fe−S cluster biosynthesis is essential for xm^5U synthesis.[43] This led Huang and co-workers to determine an elegant, radical SAM-based mechanism for Elp3-catalyzed uridine carboxymethylation.[44] Isotopic labeling experiments using an archael Elp3 supported a mechanism in which tRNA carboxymethylation proceeds via an initial SAM radical-mediated abstraction of the acetyl-CoA C2-hydrogen, followed by C−C bond formation with uridine C5-hydrogen. Subsequent hydrolysis of the uridine-acetyl-thioester intermediate yields the final 5-carboxymethyluridine product

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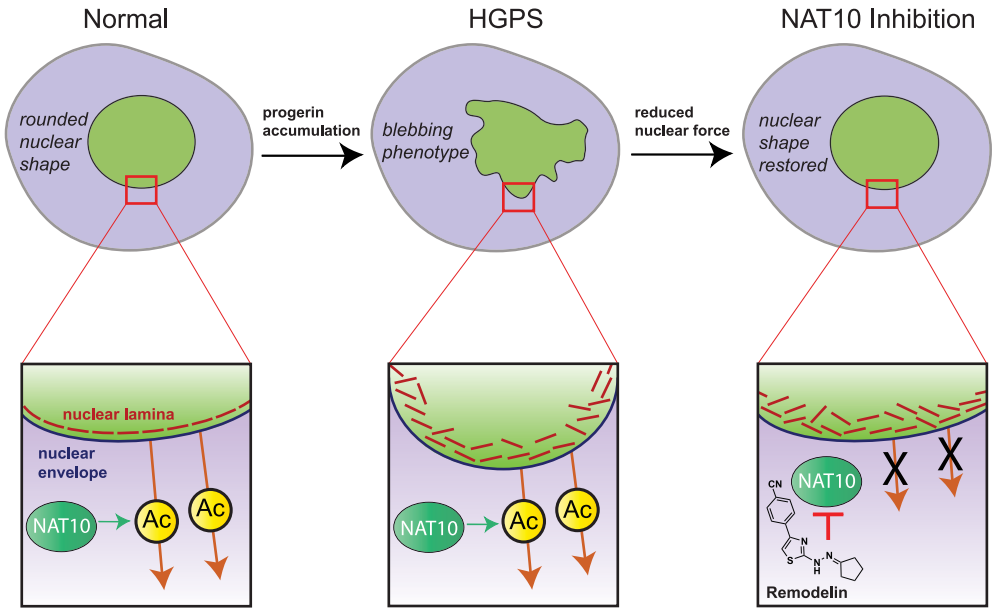

Figure 4. Role of the orphan acetyltransferase Nat10 in regulation of nuclear shape in laminopathic disorders. Left: Nat10-catalyzed acetylation contributes to external forces exerted on the nuclear membrane. Middle: In HGPS cells, an altered form of nuclear lamin (progerin) accumulates, resulting in abnormal susceptibility to external forces and defects in nuclear shape (known as blebbing). Right: Inhibition of Nat10 by a small molecule (remodelin) reduces acetylation-dependent external nuclear forces, thereby restoring normal nuclear shape.

(Figure 3b). The authors also observed that Elp3 alone hydrolyzed acetyl-CoA, potentially indicative of a covalent acetyl-enzyme intermediate. Notably, analogous acetyl-enzyme intermediates have been proposed to be critical in lysine acetyltransferase reactions.[45]

Elp3 represents a fascinating example of an orphan KAT with multiple distinct biological functions. However, a number of questions remain. For example, Huang and co-workers note that Elp3, but not other members of the Elongator complex, is highly conserved among many lower organisms and viruses.[44] This suggests Elp3's RNA carboxymethylation function may have arisen relatively early in evolution, before later being co-opted for protein lysine acetylation. As noted above, in yeast, tRNA-dependent effects of Elp3 appear to lie upstream of at least some histone acetylation events. However, in human cells, Elongator can be found associated with the coding regions of many genes.[35] These contrasting observations raise the question of whether Elp3 functions primarily as a KAT or an RNA carboxymethyltransferase in higher organisms. An important step toward unraveling this dichotomy will be to map the molecular targets of the xm^5U modifications in eukaryotic RNAs. In this respect, it is remarkable that pseudouridinylation, another primarily tRNA associated post-transcriptional modification, was recently found in diverse messenger and noncoding RNAs when interrogated by next-generation sequencing.[46,47]

In addition to Elp3, homologues of Nat10—another eukaryotic protein acetyltransferase—have been demonstrated to catalyze post-transcriptional acetylation of tRNA and rRNA cytidine residues.[48] One broad implication of these studies is that protein and nucleic acid modifications may be functionally linked to a greater degree than is currently known. For example, Fe(II)-ketoglutarate-dependent dioxygenase family members catalyze removal of methyl groups from protein, RNA, and DNA.[49−51] However, to our knowledge, the ability of any single dioxygenase to catalyze both protein and DNA oxidation has not been reported. Interestingly, in addition to its role in protein and RNA modification, Elp3 knockdown has also been reported to facilitate epigenetic reprogramming of paternal DNA demethylation in mice.[52] Mutational analyses indicate this function requires the active radical SAM domain, while the HAT domain is dispensable. Understanding the molecular basis for the integration of these multiple activities will be an important area of future research.

A CYTOSKELETAL ORPHAN ACETYLTRANSFERASE: Nat10

Another acetyltransferase that appears to exhibit multiple, context-dependent functions is the nucleolar acetyltransferase Nat10. Human Nat10 (not to be confused with the N-terminal acetyltransferase Naa10) was first identified as a histone acetyltransferase facilitating transcriptional coactivation of the telomerase gene.[53] Functional studies demonstrated a truncated Nat10 construct was able to catalyze transfer of ^{14}C-acetyl-CoA to histones *in vitro*. Specific sites of acetylation were investigated in a subsequent study that demonstrated a role for Nat10 in reformation of the nuclear envelope following mitosis.[54] Knockdown of Sun1, a factor involved in Nat10-chromosomal targeting, primarily reduced acetylation of histones H2B (K12 and K15) and H4 (K8, K12, K16). Nat10 acetylation of these sites was proposed to facilitate chromatin decondensation following cell division, priming chromatin for gene expression during interphase.[54]

In addition to histone acetylation, evidence suggests Nat10 also harbors tubulin acetyltransferase activity (Figure 4). Tubulin lysine acetylation is a highly conserved post-translational modification associated with stable, long-lived microtubules and has been shown to play a role in cell migration and neuronal function in higher organisms.[55] Following up on the involvement of Nat10 in mitosis, Zhang and co-workers found Nat10 exhibited cell-cycle dependent localization to the midbody, a tubulin-rich structure transiently formed near the end of cytokinesis just prior to cell division.[56] Depletion of Nat10 also blunted the induction of tubulin acetylation and

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impaired cytokinesis. While biochemical acetyltransferase activity was not demonstrated, these data suggest Nat10 may directly mediate tubulin acetylation. This finding is intriguing for a number of reasons. First, other studies have identified an alternative acetyltransferase, αTAT1, as the major tubulin acetyltransferase in higher organisms.[57,58] The degree to which these activities are redundant or context-dependent remains to be established. Second, since tubulin acetylation facilitates tubulin stability and reduced cellular motility, restoration of tubulin acetylation using HDAC inhibitors has been actively explored as an approach to limit cancer cell metastasis.[59] However, Nat10 is upregulated in a number of cancers and is nonintuitively associated with increased cellular motility and invasion in colorectal cancer.[56,60] This correlates with the recent finding that inhibition of αTAT1 can reduce tumor cell migration,[61] and suggests the dynamics, rather than overall balance, of tubulin acetylation may be most important for proliferation and invasion.

Along with cancer, therapeutic targeting of Nat10 has recently been proposed for treatment of Hutchinson−Gilford progeria syndrome (HGPS), a premature-aging disease.[62] HGPS and related disorders result from mutation of the gene *LMNA*, which encodes the nuclear membrane proteins lamin A and C.[63] These mutations cause production of progerin, a truncated lamin A precursor. Unlike lamin A, progerin remains anchored to the nuclear membrane, causing the nucleus to be abnormally susceptible to external forces. This manifests as a number of overt phenotypes, including misshapen nuclei and loss of repressive chromatin domains. The latter characteristic led Jackson and co-workers to examine a small panel of nine previously characterized KAT and HDAC inhibitors in a cell-based model of lamin deficiency to determine their effect on nuclear morphology.[62] Only one molecule demonstrated significant rescue of the laminopathic nuclear shape defect, a small molecule previously identified as an inhibitor of the acetyltransferase Gcn5.[64] Chemical derivatization of the small molecule with a biotin affinity handle and subsequent pulldown from cell lysates identified a number of prospective molecular targets, including lamin C, several RNA-binding proteins, and the acetyltransferase Nat10. This prompted a series of follow-up experiments in which it was rigorously demonstrated that a functional Nat10 acetyltransferase domain is required for maximal induction of lamin-dependent nuclear shape defects in model systems and HGPS patient-derived cells. A derivative of the initial small molecule hit, "remodelin" (Figure 4), was shown to reduce DNA damage and cell senescence phenotypes normally observed in HGPS cell lines.[62] Overall, these studies led to the hypothesis that Nat10-catalyzed acetylation stabilizes microtubule anchorage, which in turn exerts an external force on the nucleus that contributes to irregular morphology in cells where progerin has aberrantly accumulated in nuclear membranes. By reducing these external forces, small molecule inhibitors of Nat10 can restore normal nuclear shape and thus provide a new therapeutic avenue for treatment of the symptoms associated with this phenotype in HGPS.

These studies highlight Nat10 as a biologically relevant orphan acetyltransferase. Efforts to target Nat10 in cancer and laminopathic disorders will be facilitated by the biochemical and molecular characterization of Nat10, including specific sites of tubulin acetylation, which remain to be determined. It will also be key to further characterize the protein partners of Nat10, such as the short isoform of the bromodomain BRD4 with which it interacts in human cancer cells.[65] Similar to Elp3, prokaryotic and eukaryotic homologues of Nat10 have also been directly implicated in RNA modification, specifically the posttranscriptional acetylation of cytidine.[48,66] This activity was recently described to regulate the biogenesis of 18S rRNA in budding yeast.[48] Notably, human Nat10 contains putative helicase and tRNA-binding domains in addition to its acetyltransferase domain and has been reported to play a role in rRNA maturation, suggesting this function may be conserved.[67] Unraveling the hierarchy of these biological functions will be crucial to determine the targetable role of Nat10 in human disease.

POTENTIAL MITOCHONDRIAL ACETYLTRANSFERASE: Gcn5L1

A major finding arising from unbiased profiling of the cellular acetylome was the discovery that a high proportion of mitochondrial enzymes are acetylated (Figure 5).[8] This was

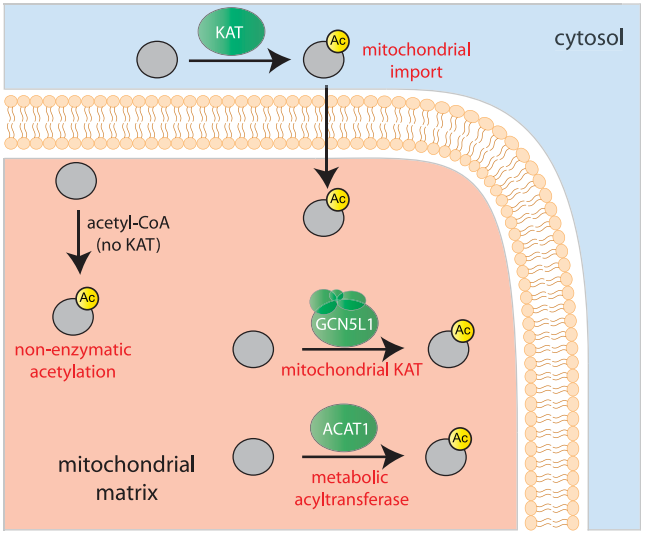

Figure 5. Mechanisms of mitochondrial protein acetylation. Hypothetical and experimental evidence suggests the following three pathways may contribute to the acetylation of mitochondrial proteins. (1) Proteins may be acetylated by nucleocytosolic KATs following protein synthesis, prior to import into the mitochondria (top). (2) Proteins may undergo nonspecific reaction with acetyl-CoA electrophiles (middle left). (3) Proteins may be acetylated by specific, mitochondrially localized KAT enzymes (bottom right).

unexpected, given that most well-studied KATs and KAT substrates had been characterized as nucleocytosolic proteins. Mitochondrial protein acetylation is functional and regulates the activity of many metabolic enzymes including those involved in fatty acid oxidation and ketone body production.[68,69] In addition, Sirt3, a mitochondrial deacetylase, can function as a tumor suppressor.[70] Thus, the question arises: how are mitochondrial proteins acetylated? Three alternative hypotheses have emerged: (1) mitochondrial proteins are preacetylated in the cytoplasm before mitochondrial import, (2) mitochondrial proteins are nonenzymatically acetylated due to the chemical conditions of the mitochondrial matrix, or (3) acetylation is driven by uncharacterized "orphan" mitochondrial acetyltransferases (Figure 5). Notably, these hypotheses are not mutually exclusive.

Regarding the first hypothesis, Hang and co-workers identified several mitochondrial proteins as putative substrates

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of p300 using a bioorthogonal reporter strategy.[27] The nucleocytosolic localization of p300 would imply these substrates undergo acetylation prior to mitochondrial import. However, these studies are far from conclusive, and overall, this potential mechanism has been largely unexplored. In contrast, prevalent nonenzymatic mitochondrial protein acetylation has been proposed by several groups. This is supported by biochemical studies showing nonenzymatic acetylation proceeds rapidly under conditions similar to those thought to exist in the mitochondrial matrix (pH 8 and millimolar acetyl-CoA levels).[71] Additionally, proteomic studies have indicated that acetyl- and succinyl-lysine acylation sites, which derive from the two most abundant mitochondrial acyl-CoAs, largely overlap, and occur with low stoichiometry.[72,73] This is consistent with a common, and potentially nonenzymatic, mechanism. It is important to note that nonenzymatic acetylation does not necessarily equate with nonfunctional acetylation. For example, nonenzymatic protein modifications can trigger important cellular processes capable of amplification (i.e., cell signaling/gene expression), as observed in signaling mediated by reactive oxygen species (ROS). Alternatively, nonenzymatic modifications may be antagonized by highly regulated programs of deacetylation and therefore have vastly different functional half-lives. A recent review comparing nonenzymatic acetylation and ROS signaling by Hirschey and co-workers provided an excellent summary of this perspective.[74]

These studies still beg the question: do specific orphan acetyltransferases that catalyze mitochondrial protein acetylation exist? To answer this question, Sack and co-workers applied an innovative bioinformatics approach to identify factors promoting acetylation of ATPase8, a protein encoded and produced wholly in the mitochondria.[75] Candidate mitochondrial KATs were identified by searching a mitochondrial protein database for proteins with homology to Gcn5 (one of the first characterized KAT enzymes). Acetylation of ATPase8 is associated with decreased oxygen consumption; therefore, knockdown of a mitochondrial KAT would be expected to increase oxygen consumption. This led to the design of an siRNA screen to assess candidate mitochondrial KATs for their ability to increase oxygen consumption. Gcn5L1 (also known as Bloc1s1) emerged as the only candidate protein whose knockdown promoted this effect. As can be inferred from the gene name, the weak homology of Gcn5L1 to yeast Gcn5 had been noted in an early study.[76] However, the primary biological role attributed to Gcn5L1 had previously been its involvement in the synthesis of lysosome-related organelles. Interestingly, while isolated Gcn5L1 exhibited sluggish KAT activity, acetylation was strongly augmented by the addition of mitochondrial extracts to Gcn5L1. This indicates Gcn5L1 may only manifest its acetylation activity when other members of its endogenous protein complex are present. Since these initial studies, Gcn5L1 has also been reported to be involved in the regulation of mitophagy.[77,78] However, due to the challenges of reconstituting its acetyltransferase activity, the biochemical substrates of Gcn5L1, as well as the degree to which they overlap with the Sirt3 deacetylation program, remain an open question.

Given the limited molecular characterization of Gcn5L1, the question remains as to whether other mitochondrial KATs may exist (Figure 5). Elp3 has been reported to localize to the mitochondria in neuronal cells,[79] potentially layering another level of complexity onto its putative protein, RNA, and DNA modification functions. In addition, recent studies have provided evidence that the primary metabolic enzymes Acat and Dlat, both of which are mitochondrially localized, may also moonlight as protein acetyltransferases (covered below). Do these enzymes catalyze mitochondrial protein acetylation, and can enzyme-catalyzed mitochondrial acetylation events be distinguished from nonenzymatic acetylation by differences in stoichiometry? Notably, the recent development of two orthogonal proteomic methods capable of directly interrogating acetylation stoichiometry[80,81] should make answering such questions feasible in the near future.

METABOLIC ENZYMES MOONLIGHTING AS KATS: ACAT1

Related to the above studies of metabolism and acetylation, very recent findings have suggested primary metabolic enzymes themselves harbor intrinsic orphan KAT activities (Figure 5). In studies of the mitochondrial pyruvate dehydrogenase complex, Chen *et al.* found that acetylation greatly reduced the activity of both PDH1, the initial enzyme component of the complex, as well as PDP1, a phosphatase that functions to stimulate PDH1 activity.[82] This reduction in pyruvate dehydrogenase complex activity, in turn, limits the conversion of pyruvate to acetyl-CoA necessary for oxidative phosphorylation by the tricarboxylic acid cycle (TCA) cycle, thereby facilitating anabolic "Warburg" metabolism and cancer cell growth in cell and xenograft models.[82] Unexpectedly, an siRNA screen revealed ACAT1 to be the crucial regulator of PDH1/PDP1 acetylation. ACAT1 is a metabolic thiolase that can catalyze either the biosynthetic condensation of two acetyl-CoA molecules to acetoacetyl-CoA, or the reverse (degradative) reaction.[83,84] *In vitro* studies confirmed the ability of recombinant ACAT1 to catalyze acetylation of purified PDH1 and PDP1. Thorough follow-up experimentation demonstrated ACAT1 was essential for pyruvate dehydrogenase-dependent glycolytic metabolism in a cell-based model of lung cancer. ACAT1 lysine acetyltransferase activity was also shown to be stimulated by growth factors and blocked by inhibitors of tyrosine-kinase mediated signaling cascades, indicative of cross talk between these two regulatory protein modifications.

While the scope and specificity of ACAT1-catalyzed protein acetylation remain to be determined, some evidence suggests the crossover between metabolic and protein acetylation may be indicative of a more general phenomenon. For example, the metabolic enzymes ACAT2 and Dlat were found to stimulate acetylation of cytosolic 6-phosphogluconate dehydrogenase (6PGDH), a critical regulator of the pentose phosphate pathway.[85] This was initially puzzling, as Dlat itself is a member of the aforementioned pyruvate dehydrogenase complex, which exhibits predominantly mitochondrial localization. However, a landmark study recently observed that in cancer cell lines, growth factor stimulation can cause nuclear translocation of the pyruvate dehydrogenase complex, whereupon it is able to directly supply acetyl-CoA for histone acetylation.[86] Growth factor signaling was also shown to stimulate Dlat-dependent acetylation of 6PGDH,[85] although further studies will be necessary to determine whether these two effects are linked. These studies provide yet another example of the rapid revisions in dogma that have come to characterize the field of catalytic acetylation and suggest further investigations into the connection between cancer metabolism and regulatory protein acetylation events are well-warranted.

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CONCLUSIONS AND FUTURE DIRECTIONS

As these examples clearly illustrate, the field of acetyltransferase biology is a vibrant and rapidly evolving one. Orphan acetyltransferases have validated links to cell and disease-associated phenotypes, and defining their functional roles in signaling represents an essential step toward targeting these enzymes therapeutically. In our view, the greatest challenge currently limiting a comprehensive view of acetyltransferase function stems from the fact that the level of molecular detail with which each acetyltransferase has been examined varies widely. This reflects shifts in the availability of experimental techniques, as well as in attitudes. Akin to the careful enzymological characterization of canonical KATs performed almost 15 years ago,[15,87] the mechanistic description of Elp3 RNA carboxymethylation illustrates the ability of biochemical insights to transform our view of novel biological function on a molecular level.[44] The relative dearth of such studies also provides a call-to-arms for chemical biologists to craft new tools enabling the rapid, functional characterization of acetyltransferase activity. To achieve maximal impact, these tools should be applicable in cellular contexts, as the discussions of p300, Elp3, and Gcn5L1 exemplify how acetyltransferase activity can differ greatly between endogenous and artificial settings. Notably, several recently developed or nascent technologies have demonstrated utility in such contexts. These include quantitative mass spectrometry techniques enabling the simultaneous monitoring of multiple histone acetylation sites,[88] new affinity probes for examining the active-site occupancy of acetyltransferases in cell extracts,[26,89] fluorescent reporters that allow live-cell visualization of protein acetylation,[90,91] and microarray and chemical genetic methods for identifying putative acetyltransferase substrates.[92,93] Such methods will benefit from the continued development of small molecule probes for KAT enzymes[94] and improved Crispr-Cas9 gene editing technologies, which will facilitate cell-based investigations of KAT activity by enabling domain specific loss of function studies. The application of high-throughput techniques for the rapid functional characterization of KAT activity is the key step necessary to bridge the knowledge gap that currently lies between canonical KATs such as p300 and Gcn5 and their less-studied orphan relatives. Assembling this knowledge will allow us to define the role of orphan KATs in acetylation-dependent signaling cascades, thereby facilitating new efforts to define the lysine acetylation program and its targetable functions in cancer and other diseases.

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Notes

The authors declare no competing financial interest.

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