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MYSTs Mark Chromatin for Chromosomal Functions

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Summary

The MYST family of lysine acetyltransferases has been intensely studied because of its broad conservation and biological significance. In humans, there are multiple correlations between the enzymes and development and disease. In model organisms, genetic and biochemical studies have been particularly productive because of mechanistic insights they provide in defining substrate specificity, the complexes through which the enzymes function, and the sites of their activity within the genome. Established and emerging data from yeast reveal roles for the three MYST enzymes in diverse chromosomal functions. In particular, recent studies help explain how MYST complexes coordinate with other modifiers, the histone variant H2A.Z, and remodeling complexes to demarcate silent and active chromosomal domains, facilitate transcription, and enable repair of DNA damage.

Keywords

SAS; Esa1; NuA4; SAS3; NuA3

Overview

The DNA coding sequence of nearly every cell in an organism is identical. However, in each cell, DNA is packaged with histones and other proteins to form chromatin, which is anything but uniform or static. Covalent modifications of chromatin provide a dynamic mechanism for controlling key cellular processes as diverse as transcription, recombination, replication, repair of DNA damage, and apoptosis. Notably, these modifications leave the underlying genomic DNA sequence intact. Among the variety of chromatin protein modifications characterized, lysine acetylation and deacetylation have been particularly well studied, initially through definition of the enzymes that control these reactions.

Among the many lysine acetyltransferases (KATs) that have been identified, the GNAT and MYST families have been intensely studied because of their broad conservation, diverse functions, and associations with metazoan development and disease (reviewed in [1-3*]). The MYST gene family was first reported in 1996, defined by its founding members in yeast and humans, *MOZ*, *YBF2/SAS3*, *SAS2* and *TIP60* [4,5].

MOZ was identified as the recurrent translocation partner in an acute myeloid leukemia, the yeast *SAS2* and *SAS3* genes were found to influence transcriptional silencing, and *TIP60* was shown to encode a human protein that interacts with the HIV-1 Tat trans-activating factor.

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These MYST family members were initially hypothesized to be histone KATs, based on limited sequence similarity to the GNATs Gcn5 and Hat1, whose enzymatic activity had been very recently defined [6-8], and to other acetyltransferases with non-histone substrates. Since these first reports, remarkable progress has been made.

Recent comprehensive reviews of yeast, fly and vertebrate MYST enzymes provide details of their substrate specificity and structure, the complexes through which they act, their biological functions, and connections to development and disease [9*-13*]. In this review, the focus will be to extend this foundation to highlight several newly emerging areas that reveal links between MYST targeting and other chromatin-modulating activities. In particular interactions between MYSTs and histone methylation and remodeling activities provide increasing mechanistic insight into how chromosomal transactions may be integrated. The recent data considered here come primarily from analysis in yeast and underscore important areas that are worthy of even broader study.

MYST Complexes and Substrate Specificity

Many of the first MYST genes were found through mutant analyses. Sequences of the cloned genes underlying the mutations allowed probing of growing genomic sequence databases and the recognition that most eukaryotes had multiple MYST representatives. These ranged from 2-3 genes in yeast species to at least 5 in vertebrates, along with splice variants whose numbers are expected to grow with further study. Early phenotypic data revealed roles for MYSTs in transcriptional silencing and sex-specific dosage compensation, and that MYST KAT activity did not merely fine-tune chromatin function, but could actually be essential for life.

In parallel with the genetic studies, biochemical purification provided an increasingly clear view of the complexes through which the MYSTs act and first glimpses of how their distinct substrate specificities influence functional output. Comprehensive structural and functional analyses of each yeast MYST complex have been reported (reviewed in [11*,14*-16*]).

Sas2 is the catalytic subunit of the simplest complex, SAS, which targets histone H4-K16 and includes the Sas4 and Sas5 subunits and targets nucleosomal histone H3. The Sas3 enzyme resides in the NuA3 complex that has at least four additional subunits. By contrast, the only essential yeast MYST protein is Esa1, and it is found in two complexes: NuA4 includes at least thirteen subunits, whereas Piccolo is a distinct sub-complex with only three subunits, all of which are shared with NuA4. Although both Piccolo and NuA4 target multiple residues of nucleosomal H4 and H2A, Piccolo is distinguished by its extremely robust activity. Details of complex purifications and information about the noncatalytic subunits of the yeast complexes are summarized in [11*,14*,17,18*].

Much of what has been learned of the yeast MYST complexes has distinct parallels in recent studies of the human and *Drosophila* complexes. Indeed, striking similarities are noted in subunit composition between the yeast and human NuA4 complexes, in which Tip60 is the catalytic subunit, Piccolo and the human HBO1 complex, and NuA3 and human MOZ/MORF complexes [9*]. Likewise, the human MSL complex with hMOF as its catalytic subunit is similar to the *Drosophila* MOF-containing dosage compensation complex, although hMOF is also found in additional complexes and is responsible for the majority of H4-K16 acetylation in the cell [19].

Beyond the biochemical purification of complexes, X-ray crystal studies of Esa1 demonstrate that its catalytic core domain and CoA interactions are nearly superimposable with those of the GNAT Gcn5, even though the two enzymes have only limited amino acid sequence similarity [10] and distinct substrate specificities. Of note is that together, Esa1 and Gcn5

appear responsible for the majority of global H4 and H3 acetylation and are generally found upstream of actively transcribed genes [20,21].

Targeting Complexes to Chromatin

Binding and reconstitution studies reveal that Esa1 targeting to substrates is mediated by the noncatalytic Epl1 and Yng2 complex subunits, which also serve to stabilize the enzyme [22-24*]. It is the histone-fold domain (HFD) regions of histones, rather than the tails themselves, that appear responsible for tight substrate binding and efficient tail acetylation [24]. Tail-independent binding was observed earlier for MOF [25] and interactions with HFDs and nucleosomal basic patches may also be relevant for Sas2 and its functions in establishing chromatin boundaries ([26*] and see below).

Many KATs, such as Esa1, must be directed to nucleosomal targets through partner proteins, yet a recent study demonstrates that is not be the case for all MYSTs. X-ray crystal structural analysis of human MOZ reveals that whereas it has a central region that is very similar to Esa1, divergent N- and C-terminal regions can strongly bind DNA [27*]. Binding is mediated through both a TFIIA-type zinc finger and a separate helix-turn-helix motif, and mutational analysis demonstrates that KAT activity and DNA binding are not mutually interdependent. It will be important to determine in future studies if specificity of MOZ targeting *in vivo* is influenced by other complex members or is controlled by the MYST domain.

In vivo Analysis of Genomic Targets

Details of residue-specific targets of MYST activity were initially defined using recombinant proteins and purified complexes. More complete understanding of genomic targets has come through directed chromatin immunoprecipitation (ChIP) and global microarray studies to monitor gene expression and genomic patterns of binding and activity *in vivo*. It is clear that each of the yeast MYSTs have distinct genomic patterns of activity, a principle likely to be shared by metazoan MYSTs as studies are expanded in those organisms.

SAS-directed acetylation of H4-K16 is important in defining boundaries between transcriptionally active and transcriptionally silenced regions of the genome. Early studies demonstrated this role in subtelomeric regions, where *sas2Δ* mutants suffered a spread of hypoacetylated histone H4 and the Sirtuin deacetylase Sir2 [28,29], which in concert with other silent chromatin components can ultimately extend the reach of heterochromatin beyond its normal limits. SAS-mediated boundary functions are also observed at other silenced regions of the genome [30,31] and increased Sas2 expression can restrict the spread of silencing by preventing binding of the Sir3 silencing protein that is ordinarily targeted to deacetylated H4K-16[26*].

NuA3's acetylation of H3 has been studied using *sas3Δ* mutants, histone mutants, epitope-tagged Sas3, and isoform specific antibodies. Results from these studies together point to a role for NuA3 in contributing to transcriptional activation of many target genes. It has recently been shown that this is a role shared by Gcn5-directed acetylation for many of the same targets [32]. Such overlapping activity is consistent with the observation that either single mutant has only modest phenotypes, whereas simultaneous loss of Gcn5 and Sas3 activity results in cell death [33], presumably due to cumulative loss of expression of genes required for cell growth and cell cycle progress. Two independent studies point to additional roles for NuA3 in chromatin boundaries [31,34], although the molecular details have not yet been as thoroughly explored as for SAS.

The essential yeast MYST Esa1 has multiple roles *in vivo*, supporting the idea that it may also have many critical genomic targets. Early analysis revealed that ribosomal protein genes,

whose expression is rate-limiting for growth, were important targets for Esa1 activation [35]. Subsequent directed and genome-wide ChIP experiments support a role for Piccolo and NuA4 in global and targeted H4 acetylation [20-22,36,37]. This acetylation contributes not only to activation, but also transcriptional silencing and repair of DNA damage [38-41*].

Less is known about the significance of Esa1-directed H2A acetylation. Genome-wide analyses suggest that in general, H2A-K7 acetylation is not associated with transcriptional activation, although Esa1-dependent acetylation is observed at the promoters of several genes examined individually [37,42]. Notably, the bromodomain protein Bdf1 binds hyperacetylated histones, including acetylated H2A-K7 in intergenic regions, and deletion of *BDF1* is lethal in an *esa1* conditional mutant background [42,43]. Bdf1 also links the acetylation state of nucleosomes modified by NuA4 to Pre-Initiation Complex (PIC) assembly and interacts with the SWR complex, which incorporates the H2A variant H2A.Z into chromatin [44*].

In fact, important recent studies define H2A.Z (encoded by the *HTZ1* gene) as an additional substrate of Esa1 [45*-47*]. H2A.Z's SWR assembly complex shares 4 subunits with NuA4 not found in Piccolo [48-50]. Mutational analysis demonstrates that H2A.Z contributes to barrier functions by contributing to restricting the spread of silencing proteins [31,45,51] and ChIP analysis demonstrates that H2A.Z influences localization of genes to the nuclear periphery and kinetics of their reactivation after repression [52*]. Several independent genomic maps of H2A.Z occupancy have been completed [47*,53-56] [41*,44*,57*]. Because H2A.Z-containing nucleosomes are found throughout the genome, understanding the regulation of their assembly and modification by Esa1 and other KATs is an area deserving further attention (see below).

Interactions between Modifications and Modifiers

The significance of one chromatin modification inhibiting or enhancing modification at another site is increasingly recognized. This concept, sometimes referred to as 'cross-talk' between modifications has been recently reviewed [3*,58*]. Specific modifications can also be influenced by ATP-dependent chromatin remodeling complexes and assembly processes. Several examples of 'cross-chromatin' modification have been recently reported for yeast MYSTs and provide new insights into their regulation and targeting (Figure 1).

SAS-directed acetylation of H4-K16 is required for efficient H2A.Z incorporation into subtelomeric chromatin [59*]. As noted above, Esa1 acetylates H2A.Z, and increased gene dosage of *SAS2* can displace the Sir3 silencing protein in telomeric regions and promote Dot1 mediated methylation of H3-K79 [26*], which functions to limit further binding and spreading of Sir3. The H3-K4 methyltransferase Set1 is also important for telomeric silencing and contributes to the restriction of Sir3p binding [60,61]. In fact, acetylated H2A.Z and Set1 act in cooperation throughout the genome, not just near telomeres, to mediate an anti-silencing protective mechanism that prevents the spread of silent chromatin into normally active regions [47*,57*]. Thus, two MYSTs acting in concert with two methyltransferases and a variant histone assembly/remodeling complex are important on both a local and genome-wide scale for restricting inappropriate silencing (Figure 1A).

To be acetylated by NuA3, histone H3 must be methylated at H3-K4 and H3-K36 [62*]. It is the PHD finger of the Yng1 component of NuA3 that binds H3K4 after it has been trimethylated and then promotes H3-K14 acetylation and subsequent transcriptional activation [62*-64*]. The mechanistic contribution of H3-K36 methylation to NuA3's KAT activity remains unknown, although it also appears linked to NuA4. In this case, deletion of the genes encoding Set2 and Set1, the H3-K36 and H3-K4 methyltransferases, respectively, are associated with decreased Esa1-dependent H4-K8 acetylation [65] (Figure 1B).

Interactions between NuA4 and SWR have also been documented with the ISW1 chromatin remodeling complex. In particular, both expression microarrays and mutant analyses indicated that these three complexes act in parallel to maintain normal growth, mediated through both activation of genes with TATA-containing promoters and repression of stress-induced genes [41*]. Another important remodeling complex, the essential RSC complex, is also functionally linked to NuA4 and SAGA complexes, both of which can stimulate SWR's effects on transcriptional elongation. In this case, it appears that RSC is recruited to nucleosomes acetylated by Esa1 and Gcn5 thereby facilitating the movement of RNA Pol II through chromatin [66*].

Finally, DNA repair and maintenance of genomic integrity are intimately linked to chromatin modification and remodeling activities (reviewed in [67*-70*]). The connection with MYST proteins was first found for Esa1 when it was discovered that the four H4 residues that it targets are critical for repair of double strand breaks [38]. NuA4 and deacetylase complexes are recruited to sites of damage [38,71,72] and dynamic (de)acetylation and (de)phosphorylation of H4 and H2A act in concert with the INO remodeling complex to effect repair [39,72,73] (Fig. 1C).

Conclusions and Outstanding Questions

This brief review has focused on yeast MYSTs, and as noted above, important progress in analysis of metazoan MYSTs has also recently been reviewed [9*-13*]. Since the discovery of the enzyme family, a broad view of MYST function through multimeric complexes has been achieved. Although we now know many of the individual histone residues on which each yeast MYST protein may act, and where in the genome, and for which cellular processes each is critical, many important questions remain to be addressed. Chief among these include defining mechanisms for regulating MYST activity, how complex assembly and disassembly may be regulated, and which additional non-histone targets may be most important biologically. Some clues to answering these questions already exist.

The form of MYST regulation that has been most deeply explored is complex targeting, with some examples as discussed above. However, it is likely that regulation of activity of the complexes may also be mediated through postranslational modification of the catalytic subunits, or other members of the complex. Already, some instances are known, notably the UV-induced sumoylation of human Tip60 [74*] that appears to enhance its catalytic activity.

Change in expression of MYST complex components is also a potential point for regulation, particularly for the components that are known to exist in more than one modifying or remodeling complex. Searching publicly available expression array datasets available for yeast (<http://www.yeastgenome.org/>) reveals environmental and genetic conditions under which MYST complex component expression is enhanced or inhibited. It may prove fruitful to pursue the effects of these conditions on both complex levels and activity.

Little is yet known about complex assembly and disassembly and whether this may be a point of regulation. One study has shown that nuclear import of SAS complex subunits is mediated through different karyopherins, demonstrating that there is likely to be no cytoplasmic pre-assembly of active complex [75]. Understanding assembly and whether subunit exchange between complexes drives activity should answer many interesting questions. For example, what dictates Piccolo vs. NuA4 assembly? Because addition of NuA4 subunits to the Piccolo core appears to decrease Esa1's KAT activity, yet enable its targeting to sites of DNA damage, it will be important to know if pools of the complexes are sensitive to damage induction.

The initial focus defining MYST substrates was on the amino terminal tails of histones, however increasingly sensitive mass spectrometry has shown that histones are modified at

many additional positions (reviewed in [76*]). Because the enzymes responsible for many of these additional sites have not yet been identified, it is possible that some or all of the MYSTs may prove to act on residues beyond those defined to date. Indeed, lysine acetylation is a prevalent posttranslational modification and new affinity based proteomic studies are being developed to define the complete spectrum of acetylated proteins [77*]. Expansion of this and parallel approaches, particularly assisted by mutant analysis should be informative. Already, candidate protein analysis has revealed that both ATM and p53 can be acetylated by Tip60 and that this acetylation is important for growth control and DNA damage response (reviewed in [78*,79*]). It has been observed that SAS complex members [80*] and NuA4 are important for mediating human p53's activation function when expressed in yeast whereas, NuA3 appears to interfere [81,82]. Thus, it appears that not only do MYST proteins likely have multiple non-histone substrates, but that further studies in yeast may be useful in dissecting complex regulatory pathways in which human MYST proteins function.

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1. . An overview of the functions and sites of action of MYST complexes throughout the yeast genome

A. Boundary Functions. At subtelomeric regions, the Sas2 MYST of the SAS complex acetylates H4-K16, thereby facilitating the SWR complex's assembly of H2A.Z-containing nucleosomes (red). H2A.Z is then acetylated by the Esa1 MYST, shown here in the context of the large NuA4 complex, which has four of the same subunits found in SWR. These events facilitate formation of heterochromatin (dark nucleosomes): euchromatin (light nucleosomes) boundaries and prevent the spread of silencing proteins such as the SIR complex (blue and green), that includes the Sir2 deacetylase (green). Methylation of H3 by Dot1 and Set1 (complexes not shown) is also important in boundary formation. Not depicted here, but recent

studies demonstrate that H2A.Z and Set1 act in concert throughout the genome, not just at subtelomeric regions, to restrict the spread of silent chromatin (see text). B. *Transcriptional Control*. Acetylation by Sas3 and Esa1 occurs throughout the genome. As part of NuA4, Esa1 targets 5' regions of genes, often sites of decreased nucleosome density. This acetylation can be important for their transcriptional activation. NuA3 also mediates transcription and targets many of the same target genes as Gcn5 (not shown). For Sas3 to acetylate H3-K14, methylation by Set1 and Set2 (not shown) must occur to facilitate targeting of NuA3 by its PHD finger component Yng1. Esa1 also acetylates much of the genome through its very active, smaller Piccolo complex. C. *DNA Damage Repair*. At sites of DNA damage, acetylation by Esa1 and NuA4 are required for repair to occur. Cycles of phosphorylation and dephosphorylation of H4 and H2A, along with the INO remodeling complex (not shown) are also important in mediating repair and recruitment of NuA4.