Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagrm

Review

# An unexpected journey: Lysine methylation across the proteome ☆

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ARTICLE INFO

*Article history:*
Received 21 October 2013
Accepted 11 February 2014
Available online xxxx

*Keywords:*
Lysine methylation
Non-histone methylation
Proteome-wide
Methylation signaling
Post-translational modification

ABSTRACT

The dynamic modification of histone proteins by lysine methylation has emerged over the last decade as a key regulator of chromatin functions. In contrast, our understanding of the biological roles for lysine methylation of non-histone proteins has progressed more slowly. Though recently it has attracted less attention, ε-methyl-lysine in non-histone proteins was first observed over 50 years ago. In that time, it has become clear that, like the case for histones, non-histone methylation represents a key and common signaling process within the cell. Recent work suggests that non-histone methylation occurs on hundreds of proteins found in both the nucleus and the cytoplasm, and with important biomedical implications. Technological advances that allow us to identify lysine methylation on a proteomic scale are opening new avenues in the non-histone methylation field, which is poised for dramatic growth. Here, we review historical and recent findings in non-histone lysine methylation signaling, highlight new methods that are expanding opportunities in the field, and discuss outstanding questions and future challenges about the role of this fundamental post-translational modification (PTM). This article is part of a Special Issue entitled: Methylation multifaceted modification — Looking at transcription and beyond.



## 1. Introduction

### 1.1. Not so new: a historical perspective on non-histone methylation

As with histone methylation, non-histone methylation was discovered long before the field was prepared to study the post-translational modification. R.P. Ambler and M.W. Rees first reported the presence of ε-N-methyl-lysine in a natural protein in flagellin from *Salmonella typhimurium* in 1959 [1]. Five years later, Kenneth Murray reported methyl-lysine in histones. Murray based his analysis in part on comparison of the unusual amino acid he found in histones to methyl-lysine from flagellin [2]. Early reports of lysine methylation focused on chemical identification of the amino acid within a small subset of proteins, including ribosomal proteins, actin, myosin and myofibrillar proteins, and cytochrome c [3–8] (reviewed in [9]). Rubisco, the most abundant protein on the planet [10], was also shown to be methylated [11].

Though today it is established that lysine methylation is a PTM, a great deal of work went into showing that the methyl group was added to lysine post-translationally, rather than directly incorporated as methyl-lysine during translation. Stocker, McDonough, and Ambler argued that methylation of flagellins occurred after lysine was incorporated into the protein because the presence of methyl-lysine was controlled by a gene other than the gene for flagellin [12]. Incorporation of radioactive methylation into methyl-lysine residues was not inhibited by puromycin, a translation inhibitor, again suggesting that methylation is a PTM [13,14]. Finally, methyl-lysine could not be conjugated to tRNA, which would be necessary if it were to be incorporated during translation [14].

If the methyl group was incorporated post-translationally, where did it come from? Using radiolabeling experiments, it was shown that either methionine or S-adenosyl-methionine could serve as the source of the methyl group on lysine [2,3,14,15]. Murray proposed that this methylation might be sequence specific [2], and by 1965, Kim and Paik argued that methylation in calf thymus nuclei was enzymatic [14]. In the years that followed, the field continued to focus on identifying new methylated proteins individually and on purifying a limited number of methylating enzymes (reviewed in [16]). As Woon Ki Paik, David C. Paik, and Sangduk Kim described, the field made little progress in identifying the biological function of this methylation [16]. Without a downstream biological outcome for lysine methylation, the broader significance of adding this moiety was unclear.

Biological insight into histone methylation was fueled by discoveries like the one from the lab of Thomas Jenuwein identifying the epigenetic regulator Suv39H1 as a histone lysine methyltransferase [17]. Notably, comparison with a known non-histone methylation pathway aided the identification of this histone methylation pathway; Rea et al. hypothesized that Suv39h1 might be a methyltransferase based on its homology to Rubisco large subunit methyltransferase [17,18]. The availability of

*Abbreviations:* PTM, post-translational modification; KMT, lysine methyltransferase; KDM, lysine demethylase; SAM, S-adenosyl methionine; MBT, malignant brain tumor; RNP, ribonucleoprotein; MS, mass spectrometry. Post-translational indications are indicated with protein name, site of modification, and modification type/extent, without spaces (for example, RelAK310me1 is monomethylation of lysine 310 on the protein RelA)

☆ This article is part of a Special Issue entitled: Methylation multifaceted modification — Looking at transcription and beyond.

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http://dx.doi.org/10.1016/j.bbagrm.2014.02.008
1874-9399/

techniques to study chromatin biology allowed quick bridging of methylation events with downstream phenotypic assays. New assays for chromatin biology grew along with the field of histone methylation—for example, chromatin immunoprecipitation (ChIP) coupled with microarrays (ChIP-chip) [19–21] and more recently ChIP coupled with deep sequencing (ChIP-seq) [22,23]. Just as Paik, Paik, and Kim pointed out that the advent of modern molecular biology opened up the long struggling field of lysine methylation in general [16], tools for studying gene regulation and chromatin naturally shifted the focus of the field toward histones and other chromatin-related proteins. Tool development, as will be discussed later, is likely to be important for studying lysine methylation on non-histone proteins as we move forward.

## 2. Key examples of non-histone methylation

Non-histone methylation has experienced a moderate renaissance with the finding that several histone lysine methyltransferases also methylate other substrates (see [24] for a recent review of lysine methyltransferases (KMTs) and corresponding histone and non-histone targets). Below we describe regulatory lysine methylation on several key non-histone proteins. As the number of lysine methylation events has greatly expanded over the last few years, this review is by no means an exhaustive discussion, but rather focuses on some specific methylation events to highlight regulatory trends and concepts.

### 2.1. p53 methylation

p53 is a central tumor suppressor and a decision node for triggering apoptosis [25], which is regulated by lysine methylation (reviewed in [24,26–28]). p53 methylation was first reported by Reinberg and colleagues. In 2004, they showed that Set7/Set9 monomethylates p53 at lysine 372 (p53K372me1) [29]. This methylation event was found to promote the stability of nuclear p53, expression of the p53 target p21, and DNA-damage induced p53-mediated apoptosis [29], potentially through preceding and promoting acetylation of p53 [30]. Several methylation events have also been reported to negatively regulate p53 activity. Smyd2 monomethylates p53 at lysine 370 [31]. This methylation event represses p53 activity by decreasing its ability to bind promoters of target genes [31]. Huang et al. proposed that K370me1 is a repressive mark due to a decrease in the fraction of total p53 bearing the mark at a target promoter upon DNA damage and because Smyd2 expression was inversely correlated with p53-target gene expression [31]. Interestingly, prior activating K372 monomethylation inhibits subsequent K370 monomethylation, but not vice versa, in part by decreasing the ability of Smyd2 to bind p53 [31]. SET8-mediated monomethylation at lysine 382 was also found to repress p53 transcriptional activation, decreasing expression of p53 target genes [32]. More recently, G9a and GLP were reported to dimethylate p53 at lysine 373 [33]. As levels of p53K373me2 do not increase with DNA damage despite a large increase in total p53 levels, this mark is postulated to repress p53 activity [33]. Consistent with this idea, knockdown of G9a and GLP increased apoptosis, with and without DNA damage [33].

p53 was the first non-histone protein for which regulatory demethylation was reported. In 2007, Berger and colleagues showed that the lysine demethylase LSD1 removes the p53K370me2 mark [34]. Dimethylation of K370 by a yet to be identified KMT activates p53 transcriptional activity through the recruitment of 53BP1, a protein involved in DNA damage signaling and initially identified as a p53 co-activator [35,36]. LSD1 demethylation of K370me2 disrupts the interaction between p53 and 53BP1 and thus acts to repress p53 function [34].

### 2.2. RelA

RelA/p65 is a component of the canonical NF-κB signaling pathway that translocates to the nucleus to bind target genes upon signaling activation. Several lysines in RelA have been reported to be methylated, including K37, K218, K221, K310, K314, and K315 [37–41]. Levy et al. provided an example of regulation of NF-κB signaling by methylation. In a biochemical screen with forty candidate KMTs, SETD6 was identified as a novel methyltransferase of the chromatin-associated fraction of the NF-κB subunit RelA [38]. SETD6-mediated methylation of RelA at lysine 310 (RelAK310me1) rendered RelA inert and attenuated RelA-driven transcriptional programs, including inflammatory responses in primary immune cells. The ankyrin repeat from GLP – an H3K9 methyltransferase involved in transcriptional repression – functioned as a recognition motif for RelAK310me1. Under basal conditions, SETD6-dependent binding of GLP to RelAK310me1 promoted a repressed chromatin state at RelA target genes through GLP-mediated H3K9 methylation. This repressive pathway was overridden by NF-κB activation-linked phosphorylation of RelA by PKCζ at serine 311 (RelAS311ph), which blocked GLP binding to RelAK310me1 and drove target gene expression [38]. These series of molecular events were the first description of a lysine methylation-signaling cascade and demonstrated a new mechanism for how integrated crosstalk between modifications on transcription factors and histones can modulate gene expression programs.

### 2.3. Dam1

Sharon Dent and colleagues have reported the Set1-dependent methylation of Dam 1 in yeast [42,43]. Dam1 forms part of a complex that regulates chromosome segregation, and is itself regulated through phosphorylation by the Aurora kinase Ipl1 [42]. Zhang et al. showed that loss of Set1 catalytic activity suppresses temperature-sensitive inactivation of Ipl1 [42]. They also found that this genetic interaction can be tied to Set1-dependent dimethylation of Dam1 at lysine 233 [42]. This methylation event interacts genetically with Ipl1 phosphorylation at nearby serine residues—K233 mutation can be rescued by subsequent mutation at S232, S234, or S235 [42]. The authors proposed that K233 methylation inhibits phosphorylation of S232 and S234 [42]. The group later showed that just as H2BK123 ubiquitination regulates Set1 methylation of H3K4, H2BK123ub is required for Set1 methylation of Dam1K233, establishing that both the histone and non-histone substrate of Set1 are regulated through the same initial histone modification pathway [43].

### 2.4. Spliceosomal proteins

The spliceosome is known to be a significant target of arginine methylation, which plays a role in snRNP assembly (see [44] for a review). Recent proteome-wide studies indicate that a significant portion of the spliceosome is also subject to lysine methylation [45–48]. The role of this methylation is as-yet unexplored. It is enticing to think that lysine methylation might modulate splicing through mechanisms such as spliceosome assembly or splice site selection, but experimental evidence in this area is currently lacking.

### 2.5. Ribosomal proteins

Methyl-lysine was discovered in ribosomal proteins of *Sacchromyces cerevisiae* in 1984 [49]. Enzymes which methylate yeast ribosomal proteins were first identified in 1989 [50]. These enzymes were dubbed M23 and M32 because they methylate Y23 and Y32, respectively [50] (the enzymes are now referred to as Rkm2 and Rkm1, methylating Rpl12 and Rpl23, respectively [51]). A number of papers from Steven Clarke and colleagues describe a series of methylation events on ribosomes and the corresponding KMTs. The KMT Rkm1 dimethylates Rpl23ab at lysine 105 and 109 [52,53] and Rkm2 trimethylates Rpl12ab, most likely at lysine 3 (potentially at lysine 10) [54,55]. Rpl42ab is monomethylated at lysines 40 and 55 by Rkm3 and ySET7, respectively [55]. Rkm5 monomethylates Rpl1K46 [56]. As discussed in [56], it is unclear whether these yeast methylation events translate

Please cite this article as: K.E. Moore, O. Gozani, An unexpected journey: Lysine methylation across the proteome, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbagrm.2014.02.008