



## Enhanced PKMT-substrate recognition through non active-site interactions

Margarita Kublanovsky <sup>a, b</sup>, Amir Aharoni <sup>b, c, 1, \*</sup>, Dan Levy <sup>a, b, 1, \*\*</sup>

<sup>a</sup> The Shraga Segal Department of Microbiology, Immunology and Genetics, Ben-Gurion University of the Negev, Be'er Sheva 84105, Israel

<sup>b</sup> The National Institute for Biotechnology in the Negev (NIBN), Ben-Gurion University of the Negev, Be'er Sheva 84105, Israel

<sup>c</sup> Department of Life Sciences, Ben-Gurion University of the Negev, Be'er Sheva 84105, Israel

### ARTICLE INFO

#### Article history:

Received 11 May 2018

Accepted 15 May 2018

Available online 24 May 2018

#### Keywords:

Lysine methylation

Protein lysine methyl-transferases

SETD6

SETD7

Molecular recognition

### ABSTRACT

Protein lysine methyltransferases (PKMTs) catalyze the methylation of lysine residues on many different cellular proteins. Despite extensive biochemical and structural studies, focusing on PKMT active site-peptide interactions, little is known regarding how PKMTs recognize globular substrates. To examine whether these enzymes recognize protein substrates through interactions that take place outside of the active site, we have measured SETD6 and SETD7 activity with both protein and peptide RelA substrate. We have utilized the MTase-Glo™ methyltransferase assay to measure the activity of SETD6 and SETD7 with the different RelA substrates and calculated the Michaelis-Menten (MM) parameters. We found an up to ~12-fold increase in  $K_M$  of the PKMTs activity with RelA peptide relative to the respective full-length protein, emphasizing the significantly higher PKMT-protein interaction affinity. Examination of SETD6 and SETD7 activity toward the same RelA substrates highlight the similarity in substrate recognition for both PKMTs. Our results show that the interaction affinity of SETD6 and SETD7 with RelA is enhanced through interactions that occur outside of the active site leading to higher catalytic efficiency and specificity. These interactions can significantly vary depending on the PKMT and the specific methylation site on RelA. Overall, our results underline that PKMTs can recognize their substrates through docking interactions that occur out of the active site-peptide region for enhancing their activity and specificity in the cellular environment.

© 2018 Elsevier Inc. All rights reserved.

## 1. Introduction

Lysine methylation of histone and non-histone proteins is catalyzed by protein lysine (K) methyltransferases (PKMTs). Currently, there are over 60 members of this enzyme family, the vast majority of which contain a conserved SET domain that is responsible for the enzymatic activity [1–3]. In the past decade PKMTs have attracted significant attention due to their roles in regulating central biological processes and defects in their activity were associated with various diseases. Current PKMT research focuses on extensive investigation of the cellular roles of methylation,

on deciphering the catalytic mechanism of PKMTs and on discovering new substrates for methylation [4–8].

In recent years, significant efforts were devoted to identifying novel substrates for different PKMTs including the development of high-throughput (HT) approaches for revealing hundreds of novel substrates [9–13]. Substrates identified by HT approaches are then used to search for a consensus recognition sequence flanking the target modification residue to enable the discovery of additional PKMT substrates. While the number of identified substrates is increasingly growing, very little is known regarding the molecular basis for PKMT-substrate recognition. Biochemical and structural approaches are currently utilized to study the PKMT-substrate recognition mainly by elucidating the interaction of these enzymes with a specific peptide region derived from the respective target protein substrate. SET Domain-Containing Protein 7 (SETD7) is a prominent PKMT that methylates multiple cellular proteins, including TATA-Box Binding Protein Associated Factor 10 (TAF10), p53, Estrogen receptor  $\alpha$  (ER $\alpha$ ), DNA Methyltransferase 1 (DNMT1), P300/CBP-associated factor (PCAF) and the V-Rel

\* Corresponding author. The National Institute for Biotechnology in the Negev (NIBN), Ben-Gurion University of the Negev, Be'er Sheva 84105, Israel.

\*\* Corresponding author. The Shraga Segal department of Microbiology, Immunology and Genetics, Ben-Gurion University of the Negev, Be'er Sheva 84105, Israel.

E-mail addresses: [aaharoni@bgu.ac.il](mailto:aaharoni@bgu.ac.il) (A. Aharoni), [ledan@post.bgu.ac.il](mailto:ledan@post.bgu.ac.il) (D. Levy).

<sup>1</sup> Both corresponding authors contribute equally.

Reticuloendotheliosis Viral Oncogene Homolog A (RelA or p65) subunit of NF $\kappa$ B leading to different physiological and cellular responses [14–20]. Similarly, analysis of the enzymatic activity and substrate specificity of the novel PKMT SET Domain-Containing Protein 6 (SETD6) revealed methylation activity toward over a hundred cellular proteins [9,21–29]. Currently, the most characterized SETD6 substrate is RelA which is monomethylated at lysine 310 (K310) [25]. The multi-specificity of SETD7 and SETD6 raises questions regarding the mechanism by which these enzymes recognize specific lysine residues within a diverse set of protein substrates. Analysis of target peptide sequences of SETD7 that were derived from the different protein substrates, revealed possible consensus recognition sequences that include [R/K - S/T/A - **K** - X] (X-any polar residue, **K** (bold)-methylation site) [30] or [G/R/H/K/P/S/T - K > R - S > (K/Y/A/R/T/P/N) - **K**] [31]. However, this short motif can be found ~100,000 times in the human proteome, suggesting that additional recognition mechanisms must exist to enable specific protein methylation in the cell. Additional analysis of SETD7 substrate sequences has shown that sequences that significantly deviate from the consensus sequence can be methylated [31]. These findings raise the possibility that PKMT-substrate recognition extends beyond the peptide-active site region leading to enhanced substrate binding affinity and specificity. Previously, kinases were shown to overcome the limited specificity of active site-target interaction by binding to regions of the substrate that are distant from the target phosphorylation site [32].

To examine whether PKMTs can interact with regions in the protein substrate that are distant from the target lysine, we examined the activity of SETD6 and SETD7 with their common RelA protein and peptide substrates. We measured SETD6 and SETD7 activity with the different RelA substrates and determined the Michaelis-Menten (MM) constants. We found that the affinity of SETD6 and SETD7 for RelA protein is up to ~12 fold higher relative to the respective peptide suggesting that, similar to kinases, PKMTs-substrate interaction can take place outside of the active-site-peptide region.

## 2. Results and discussion

### 2.1. Generation of RelA derived truncations to study SETD6 and SETD7 mode of recognition

To examine SETD6- and SETD7-RelA interactions, we generated a series of RelA derived truncations: RelA-FL (residues 1–431), RelA N' (residues 1–321), RelA C' (residues 300–431) and RelA peptide (residues 302–316) (Figs. 1A and 2A). Each truncation places the target residue for SETD6 (K310) or SETD7 (K314, K315) at one terminus of the protein allowing the examination of the C- or N-terminal domain contribution to PKMT activity. The truncated proteins were expressed in *E. coli* in a similar manner and we verified that both PKMTs methylate these substrates in the presence of <sup>3</sup>H-labeled SAM *in vitro* (Sup. Fig. 1A and B).

### 2.2. Regions beyond the methylation site enhance RelA recognition by SETD6 and SETD7

To determine the MM parameters for SETD6 toward the different RelA derived truncations, we monitored the methylation rate at different substrate concentrations using the MTase-Glo™ luminescence coupled assay. This assay allows monitoring the time dependent formation of S-adenosyl homocysteine (SAH) for full MM analysis (Fig. 1). The determined  $K_M$  value for SETD6 with the full-length substrate (RelA FL) was 16.63  $\mu$ M which is similar to the  $K_M$  measured with the two RelA truncated proteins (11.95  $\mu$ M and 16.15  $\mu$ M for RelA C' and RelA N', respectively (Fig. 1D–E)). In

contrast, the  $K_M$  value for the RelA derived peptide was 55.15  $\mu$ M (Fig. 1F) that is 3–5-fold higher than the  $K_M$  for RelA FL. These results demonstrate the significantly higher affinity of SETD6 for RelA protein relative to the peptide substrate suggesting that additional RelA protein recognition sites are located outside of the active site at K310.

Next, we measured the methylation rate of SETD7 toward the different RelA substrates (Fig. 2). We found that the  $K_M$  for the full-length protein is 14.43  $\mu$ M (Fig. 2C), which is similar to the  $K_M$  obtained for SETD6 activity with this substrate. In contrast, the  $K_M$  values of SETD7 for RelA N' (27.73  $\mu$ M, Fig. 2D) and RelA C' (40.17  $\mu$ M, Fig. 2E) are ~2 and ~3-fold higher than for RelA FL, respectively. Strikingly, we found that the  $K_M$  value for RelA derived peptide was more than 10-fold higher than for RelA FL (Fig. 2F). These results suggest that, similar to SETD6, interactions between SETD7 and RelA take place outside of the active-site peptide region.

### 2.3. Distinct recognition properties of SETD6 and SETD7 toward RelA

In order to further evaluate the activity of SETD6 and SETD7 with RelA peptide and protein, we compared their  $k_{cat}$  parameters for these substrates. We found a considerable increase in the  $k_{cat}$  values obtained with RelA C' and RelA peptide substrates relative to the RelA FL protein. Specifically, we found a ~12-fold and ~15-fold increase, respectively, for SETD6 and ~13-fold and ~63-fold, respectively, for SETD7 (Table 1 and Sup. Fig. 2 for graphs). The catalytic rate ( $k_{cat}$ ) can be affected by small movement of the catalytic residues with respect to the SAM and the lysine acceptor inside the catalytic site. As RelA peptide can be highly flexible, it may allow better positioning of the target lysine residue for catalysis, relative to RelA FL. This can explain the increase in the  $k_{cat}$  values of both SETD6 and SETD7. Previously, SETD7 was shown to have a higher  $k_{cat}$  values toward a H3 peptide substrate relative to the respective protein *in vitro* [17,30].

Our results highlight that while the  $K_M$  and  $k_{cat}$  of SETD6 and SETD7 toward the RelA FL protein substrate are similar (Table 1), the respective values for the RelA peptide vary significantly. These results suggest that PKMTs can recognize their substrates through interactions that occur out of the active site-peptide region (Sup. Fig. 3) for enhancing their activity and specificity in the cellular environment. Previously, recognition mechanisms that occur outside of the enzyme active site were extensively studied in kinases [33,34]. Many protein kinases can recognize a very promiscuous peptide sequence, yet they are able to recognize a range of several to hundreds of specific substrates out of ~700,000 potential substrates [32,35]. This high level of substrate specificity is achieved by employing a wide variety of recognition and interaction mechanisms that take place out of the catalytic pocket such as different types of distal docking sites, targeting subunits and scaffolds [32,36,37]. These recognition mechanisms were shown to enhance the catalytic activity and specificity of these enzymes providing novel means for kinase inhibition. Our findings showing that interactions that occur outside of SETD6 and SETD7 active site significantly contribute to methyl-transfer catalysis to RelA, pave the way for additional molecular analysis of PKMT-substrate recognition for better analysis of PKMTs methylation network.

## 3. Materials and methods

### 3.1. Molecular biology

SETD6 was expressed in pET-Duet plasmid containing poly-histidine (6xHis) tag fused to the gene. SET7/9 gene was cloned into

Download English Version:

<https://daneshyari.com/en/article/8292541>

Download Persian Version:

<https://daneshyari.com/article/8292541>

[Daneshyari.com](https://daneshyari.com)