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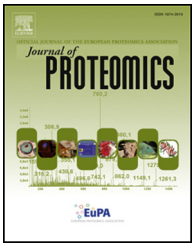

Methylation of the DNA/RNA-binding protein Kin17 by METTL22 affects its association with chromatin

Philippe Cloutier[a], Mathieu Lavallée-Adam[b], Denis Faubert[a], Mathieu Blanchette[b], Benoit Coulombe[a,c,*]

[a]Institut de Recherches Cliniques de Montréal (IRCM), Montréal, Québec H2W 1R7, Canada
[b]McGill Centre for Bioinformatics and School of Computer Science, McGill University, Montréal, Québec H3A 2B4, Canada
[c]Department of Biochemistry, Université de Montréal, Montréal, Québec H3T 1J4, Canada

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ABSTRACT

Kin17 is a protein that was discovered through its immunoreactivity towards an antibody directed against prokaryotic RecA. Further study of Kin17 revealed a function in DNA replication and repair, as well as in pre-mRNA processing. Recently, it was found that Kin17 is methylated on lysine 135 by the newly discovered methyltransferase METTL22. To better understand the function of Kin17 and its regulation by methylation, we used multiple cell compartment protein affinity purification coupled with mass spectrometry (MCC-AP–MS) to identify novel interaction partners of Kin17 and to assess whether these interactions can take place on chromatin. Our results confirm that Kin17 interacts with METTL22 both in the soluble and chromatin fractions. We also show that many RNA-binding proteins, including the previously identified interactor BUD13 as well as spliceosomal and ribosomal subunits, associate with Kin17 in the soluble fraction. Interestingly, overexpression of METTL22 in HEK 293 cells displaces Kin17 from the chromatin to the cytoplasmic fraction, suggesting a role for methylation of lysine 135, a residue that lies within a winged helix domain of Kin17, in regulating association with chromatin. These results are discussed in view of the putative cellular function of Kin17.

Biological significance
The results shown here broaden our understanding of METTL22, a member of a family of newly-discovered non-histone lysine methyltransferases and its substrate, Kin17, a DNA/RNA-binding protein with reported roles in DNA repair and replication and mRNA processing. An innovative method to study protein–protein interactions in multiple cell compartments is employed to outline the interaction network of both proteins. Functional experiments uncover a correlative role between Kin17 lysine methylation and its association with chromatin.

1. Introduction

The RecA gene is universally conserved among prokaryotes. It encodes a protein that binds both single-stranded and double-stranded DNA and is involved in homologous recombination. RecA is therefore essential in maintaining genome stability as a mediator of the DNA damage-resolving pathway known as the SOS response.

Antibodies raised against prokaryotic RecA have been shown to cross-react with nuclear proteins in mammalian

* Corresponding author at: Gene Transcription and Proteomics Laboratory, Institut de recherches cliniques de Montréal (IRCM), 110 avenue des Pins Ouest, Montréal, Québec H2W 1R7, Canada. Tel.: +1 514 987 5662; fax: +1 514 987 5663.
E-mail address: benoit.coulombe@ircm.qc.ca (B. Coulombe).

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cells. These proteins are more abundant in proliferating cells and are upregulated by genotoxic agents [1]. Screening of a mouse embryo cDNA library for gene products that display affinity towards RecA antibodies has lead to the discovery of one such gene, which was given the generic name "immunological kinship to RecA protein clone 17" (Kin17; official gene name KIN). Unlike the RAD51 family of proteins, Kin17 shares very little sequence homology with RecA. However, Kin17 does have a number of features that point to a possible role in DNA repair. For instance, Kin17 has the ability to bind to curved DNA [2], a structure known to arise at illegitimate recombination junctions. Ionizing UVC and γ radiations have also been shown to upregulate Kin17 expression and trigger formation of Kin17-containing nuclear foci associated with chromatin [3–5]. This upregulation appears to be dependent upon the global genome repair pathway, as primary fibroblasts from *xeroderma pigmentosum* (XP) patients with inactivated XPA and XPC genes are unable to promote Kin17 expression following UVC exposure [6]. Yet another element linking Kin17 to DNA repair comes from the observation that RKO cells with impaired Kin17 expression exhibit a six-fold increase in radiosensitivity compared to their wild type counterparts [7].

DNA repair factors often act in concert with chromatin-bound complexes involved in other aspects of DNA metabolism, such as RNA and DNA polymerases. Unsurprisingly, Kin17 was also shown to have a broader role in DNA replication. It was observed that the cell cycle was arrested in S-phase both in Kin17-depleted RKO cells [5] and in cells where Kin17 was ectopically overexpressed [8,9]. In SV40-transformed cells, Kin17 expression is upregulated and the protein interacts with the T antigen, the major viral replication factor of SV40. Kin17 has an inhibitory effect on T antigen-mediated replication. This effect is not limited to viral replication, but applies to cellular DNA synthesis as well, since ectopic overexpression of Kin17 in HeLa cells was noted to negatively impact bromodeoxyuridine uptake [8]. Electron microscopy imaging demonstrates colocalization of Kin17 with replication factors RPA70, PCNA and DNA polymerase α, while Kin17-directed chromatin immunoprecipitation shows association with DNA replication origins during the G1/S transition as well as throughout S-phase [10], further hinting at a probable role in DNA replication.

Structural analyses of the 45 kDa protein encoded by the Kin17 gene have identified numerous features that may also provide insight into its function (for a summary of Kin17 structure, see Fig. 1). On the N terminus of Kin17 resides a C_2H_2 zinc-finger domain (residues 28–50) with dual affinity for DNA and RNA [2,11]. In fact, there has been growing evidence for an alternate role for Kin17 as an RNA-binding protein [12]. Furthermore, the identification of Kin17 in spliceosomal purifications may imply a possible role in mRNA processing [13,14]. A separate RNA-binding module exists in the Kin17 C-terminal region (residues 268–393) with dual SH3-like domains containing a KOW motif [11]. Strangely, although Kin17 is generally conserved, this segment is absent in lower eukaryotic orthologs like Rts2p in *Saccharomyces cerevisiae*.

Between these two domains lies a region (residues 71–281) that mediates binding to curved DNA [2] and contains the antigenic determinant for the RecA antibody [15]. The only discernable structural feature within Kin17's middle section is an uncommon winged helix (residues 51–160). Although winged helix domains are typically mediators of DNA interactions (the forkhead transcription factor family constitutes a well-known example of this; [16]), it was noted that the positioning of the recognition helix as well as the electrostatic potential surface are both inadequate for nucleic acid recognition and as such the winged helix domain of Kin17 may be involved in protein–protein transactions [17].

Earlier this year, a new family of putative methyltransferases with distant homology to protein arginine methyltransferases (PRMTs) was uncovered [18]. Affinity Purification coupled with tandem Mass Spectrometry (AP–MS) was used to identify potential substrates as well as regulators of the

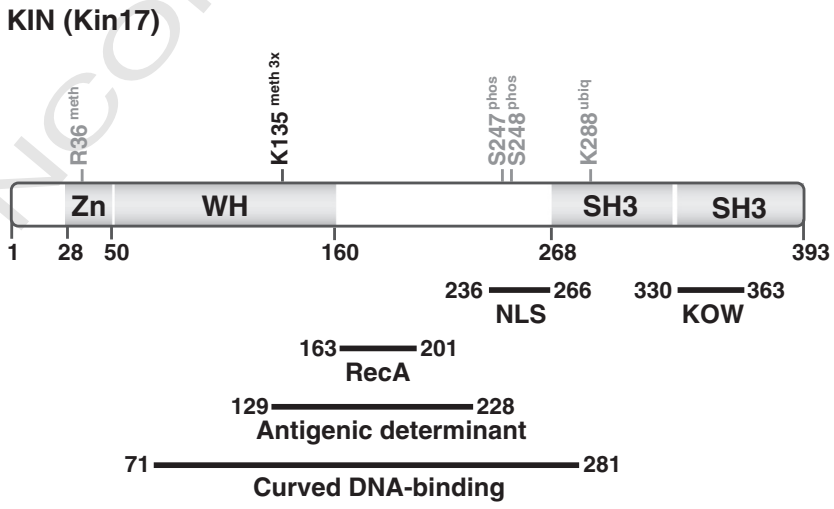

Fig. 1 – Linear representation of Kin17 domain architecture. Residues delineating each domain are indicated below. Zn, Zinc finger; WH, Winged Helix; SH3, Src Homology 3. Regions corresponding to the nuclear localization signal (NLS), KOW motif, RecA homology, RecA antigenic determinant and curved DNA/binding property are shown below. Positions of modified residues identified by more than one spectrum in the PhosphoSitePlus® database are shown above.

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