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Activity and specificity of the human SUV39H2 protein lysine methyltransferase

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ABSTRACT

The SUV39H1 and SUV39H2 enzymes introduce H3K9me3, which is essential for the viability of mammalian cells. It was the aim of the present work to investigate the substrate specificity and product pattern of SUV39H2. Methylation of peptide SPOT arrays showed that SUV39H2 recognizes a long motif on H3 comprising T6–K14, with highly specific readout of R8, S10, T11 and G12 and partial specificity at T6, A7, G13 and K14. Modification of R8 and phosphorylation of S10 or T11 lead to a reduction or loss of SUV39H2 activity towards H3K9. The specificity of SUV39H2 differs from other H3K9 PKMTs, like Dim-5 or G9a, and these biochemical differences can be explained by the structures of the corresponding enzymes. Based on the specificity profile we identified additional non-histone candidate substrates in human proteins, but all of them were only weakly methylated by SUV39H2 at the peptide level. We conclude that SUV39H2 displays a high preference for the methylation of H3. Using the catalytic SET domain we show here that the enzyme prefers H3K9me0 as a substrate over H3K9me1 and H3K9me2 and it introduces the first two methyl groups into H3K9me0 in a processive reaction. SUV39H2 can transfer up to three methyl groups to lysine 9 of histone H3 but the last methylation reaction is much slower than the first two steps. We also demonstrate that the N324K mutant in the SET domain of SUV39H2 that has been shown to cause an inherited nasal skin disease in Labrador Retrievers renders SUV39H2 inactive. Differences in the circular dichroism spectra of wild type and mutant proteins indicated that the mutation causes slight structural changes.

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1. Introduction

The unstructured N-terminal tails of histones protrude from the core nucleosome and harbor complex patterns of post-translational modifications (PTMs) including lysine and arginine methylation, lysine acetylation and phosphorylation of serine and threonine [1–4]. These PTMs regulate chromatin biology and gene expression and play a central role in the development of multicellular organisms. In addition, they are implicated in many diseases, such as cancer [5,6]. Although known since 50 years [7], the essential biological role of histone lysine methylation was first discovered in 2000 by Rea et al. showing that trimethylation of histone 3 at lysine 9 is an essential mark in heterochromatin formation [8]. In the same paper, it was also reported that this mark is introduced by enzymes of the Suv39 family, which has two members in the human genome, SUV39H1 and SUV39H2 and the enzymatic activity of murine Suv39h1 was demonstrated. The enzymatic activity of murine Suv39h2 towards methylation of H3 at K9 was shown later as well [9]. SUV39 proteins and their function in heterochromatin formation are evolutionarily highly conserved and orthologous proteins of SUV39H1 and SUV39H2 can be detected in many organisms from fission yeast to humans including plants ([10,11] and references therein). For example, the Suv39 homolog in *Schizosaccharomyces pombe* (Clr4) also plays a role in heterochromatin formation [12]. SUV39H1 and SUV39H2 both consist of two conserved chromatin domains, one SET and one Chromo domain. The SET (Su(var)3-9, Enhancer-of-zeste, Trithorax) domain is the catalytic center of most of protein lysine methyltransferases (PKMTs) [13] and Chromo domains are methylated lysine recognizing modules [14]. While the SUV39H1 Chromo domain was shown to recognize H3K9me2/3 [15], the function of the SUV39H2 Chromo domain is not yet defined. In *S. pombe*, the H3K9me2/3 binding of the Clr4 Chromo domain was shown to have a role in heterochromatin spreading [12,16]. The structure of the SUV39H2 SET domain has been solved and it shows high similarity to other H3K9 PKMTs like Dim-5 or G9a [17].

While *Suv39h2* knock-out mice are viable, deletion of both *Suv39h1* and *Suv39h2* is lethal [8,18]. It resulted in a drastic loss of pericentric H3K9 trimethylation and also led to chromosomal instabilities [18–20]. *In vivo*, both Suv39h1 and Suv39h2 introduce H3K9me3 at pericentric heterochromatin, as shown by the finding that the reduction of heterochromatic H3K9 trimethylation in Suv39 double knock-out cells was efficiently recovered by ectopic expression of either Suv39h1 or Suv39h2 [20]. Suv39h1 has been shown to introduce trimethylation at H3K9

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in vitro, but the conversion of H3K9me2 into the trimethylated state was slow [21]. A steady-state analysis with SUV39H2 indicated that the unmethylated peptide was preferred as substrate over monomethylated and dimethylated peptides [22]. Specificity analyses with SUV39H2 showed that the mutation of H3K4 and posttranslational modifications of the H3 tail amino acids like K14 acetylation and S10 phosphorylation reduce its catalytic activity [9]. The expression profiles of Suv39h1 and Suv39h2 in mice are overlapping in embryogenesis, but Suv39h2 remained expressed in adult testis and it is localized at meiotic heterochromatin. Based on these observations murine Suv39h1 and Suv39h2 have overlapping functions, but Suv39h2 has additional roles in the organization of meiotic heterochromatin [9]. However, SUV39H2 has also been connected with specific effects. It has been shown that stress induced H3K9 methylation in the hippocampus was correlated with an upregulation of SUV39H2, suggesting that the enzyme plays a functional role in this process [23]. Moreover, a SUV39H2 N324K mutation has been identified to cause hereditary nasal parakeratosis in Labrador Retriever dogs [24], which is a monogenic, inherited, autosomal recessive skin disorder. Defects in the differentiation of the specialized nasal epidermis cells in affected dogs lead to the formation of crusts and fissures in the nasal planum already present at young age, while the animals otherwise are healthy. Based on the genetics of the disease and the strong conservation of N324 in SUV39H2 enzymes from various species, Jagannathan et al. [24] predicted that the N324K mutation disrupts the activity of the enzyme but this has not been verified so far.

Despite its important biological role, not much is known about the enzymatic properties and specificity of SUV39H2. The aim of this work was to express and purify the full length and SET domain of human SUV39H2 and further characterize its substrate specificity and product pattern and investigate if it has additional substrates. Furthermore, we also aimed to characterize the properties of the SUV39H2 N324K disease mutation in dogs.

2. Results

2.1. Activity and specificity of SUV39H2

Human SUV39H2 was cloned as His_6 fusion protein and its SET domain was cloned as His_6 and GST fusion proteins (Fig. 1A). All proteins were expressed in *Escherichia coli* and purified by affinity chromatography (Fig. 1B). The GST-SUV39H2-SET domain could be obtained at high micromolar concentrations with good purity, but in spite of several attempts the preparations of the other enzyme versions still contained some contaminations and the enzymes were present only at lower concentrations. To determine their methylation activity, recombinant histone H3 was incubated with same concentrations of the purified SUV39H2 enzymes in the presence of radioactively labeled AdoMet. Afterwards the reaction mixture was separated by SDS polyacrylamide gel electrophoresis and the amount of radioactivity transferred to H3 was analyzed by autoradiography. As shown in Fig. 1C, the purified GST-SUV39H2-SET was highly active in methylating recombinant histone H3. The His-tagged version of SUV39H2-SET and the full length SUV39H2 showed a much lower activity. Next we aimed to investigate the specificity of SUV39H2 for its substrate peptide. For this we used peptide SPOT arrays, which contain peptides covalently bound to a cellulose membrane that can be directly methylated by PKMTs using AdoMet with radioactively labeled methyl groups. After washing, the radioactivity transferred to the arrays is visualized by autoradiography. Peptide arrays are ideal tools for the specificity analysis of PKMTs, because methylation of many different peptide substrates can be

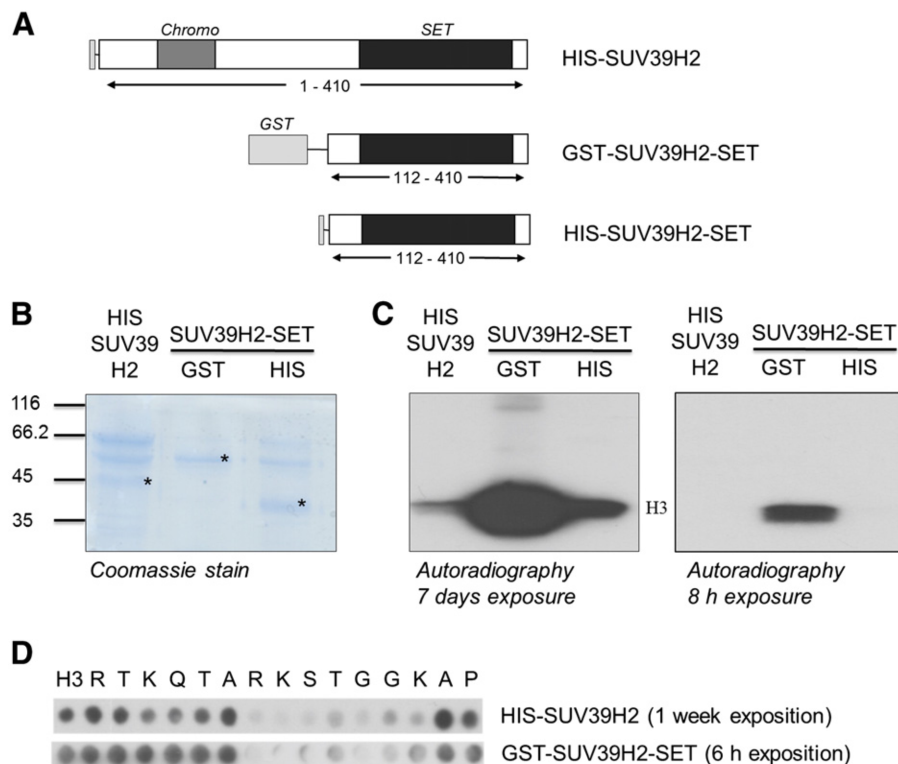

Fig. 1. Purification and initial characterization of SUV39H2 fusion proteins. A) Schematic picture of the constructs used in this work. B) Purification of the full length His_6-tagged SUV39H2 enzyme and its SET domain in GST and His_6-tagged form. The image shows a Coomassie stained SDS gel of the purified proteins. C) H3 methylation activity of the different SUV39H2 fusion proteins. H3 was incubated with the enzymes in the presence of radioactively labeled AdoMet, and subjected to SDS gel electrophoresis. The figure shows autoradiographic images of the resulting SDS gel after different exposure times of the X-ray film. The position of the H3 band is indicated. D) Sequence specificity of SUV39H2 and SUV39H2-SET. SPOT peptide arrays were prepared to contain the H3 2–16 peptide (first spot on the left side labeled with H3) together with an alanine scan of this region in which each residue was individually exchanged to alanine (following spots from left to right). The sequences of the A7 and A15 spots are identical to the original H3 sequence. The array was incubated with the SUV39H2 enzymes in the presence of radioactively labeled AdoMet. The figures show autoradiographic images of the methylated peptide SPOT array. The exposition time of the film is indicated.

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