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Research paper

Regulation of the human Suv3 helicase on DNA by inorganic cofactors

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ABSTRACT

Mitochondria are essential organelles and consequently proper expression and maintenance of the mitochondrial genome are indispensable for proper cell function. The mitochondrial Suv3 (SUPV3L1) helicase is known to have a central role in mitochondrial RNA metabolism and to be essential for maintenance of mitochondrial DNA stability. Here we have performed biochemical investigations to determine the potential regulation of the human Suv3 (hSuv3) helicase function by inorganic cofactors. We find that hSuv3 helicase and ATPase activity *in vitro* is strictly dependent on the presence of specific divalent cations. Interestingly, we show that divalent cations and nucleotide concentration have a direct effect on helicase substrate stability. Also, hSuv3 helicase is able to utilize several different nucleotide cofactors including both NTPs and dNTPs. Intriguingly, the potency of the individual nucleotide as energy source for hSuv3 unwinding differed depending on the included divalent cation and nucleotide concentration. At low concentrations, all four NTPs could support helicase activity with varying effectiveness depending on the included divalent cation. However, at higher nucleotide concentrations, only ATP was able to elicit the helicase activity of hSuv3. Consequently, we speculate that the capacity of hSuv3 DNA unwinding activity might be sensitive to the local availability of specific inorganic cofactors.

1. Introduction

Mitochondria are essential organelles serving multiple roles, which are indispensable for normal cell function [1]. According to the Warburg hypothesis, a driving force in tumourigenesis is a change in cell energy metabolism due to insufficient mitochondrial function. Recently, maintenance of the mitochondrial DNA (mtDNA) was causatively linked to cancer suppression in the mouse model of the mitochondrial helicase Suv3 [2]. Heterozygous ($mSuv3^{+/-}$) mice were found to be predisposed to cancer, a phenotype which was aggravated through each generation. The pathologies were passed maternally to both offspring with heterozygous ($mSuv3^{+/-}$), but also homozygous ($mSuv3^{+/+}$) *SUV3* gene status. In addition, cohort studies of human breast cancer patients found the expression of human Suv3 (hSuv3) to be reduced in the malignant cells compared to the normal cells. Accordingly, hSuv3 was classified as a tumor suppressor [2].

Originally, the Suv3 protein was described to play a role in mitochondrial RNA metabolism, where it forms the degradosome complex in association with a nuclease [3–5]. In *Saccharomyces cerevisiae* and *Trypanosoma brucei* the nuclease is believed to be Dss1p, while in humans it is believed to be PNPase [4–8].

In addition, a potential role for Suv3 in DNA metabolism has been speculated. Loss of Suv3 is associated with a decrease in mtDNA copy-number in yeast, mice and human cells [2,9,10]. Inactivation of Suv3 in mice results in an increase in mtDNA mutations [2]. *In vitro*, hSuv3 has a preference for dsDNA over RNA substrates [11] and in *S. cerevisiae* Suv3 was found to associate with active origins of

Abbreviations: bp, basepair; BSA, bovine serum albumin; dNTP, deoxynucleoside triphosphate; dsDNA, double stranded DNA; Dss1p, deletion of Suv3 suppressor 1; FEN1, flap endonuclease 1; mtDNA, mitochondrial DNA; mtRNA, mitochondrial RNA; NTP, nucleoside triphosphate; OXPHOS, oxidative phosphorylation; PNPase, polynucleotide phosphorylase; Polγ, polymerase gamma; ssDNA, single stranded DNA; Suv3, suppressor of Var1 − 3; T_m, DNA melting temperature; WRN, Werner protein.

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replication, without the Dss1p nuclease, suggesting a potential direct role in mtDNA replication [10]. In addition to the major mitochondrial function, a small fraction of hSuv3 localizes to the nucleus [12–14]. In the nucleus, knockdown of hSuv3 results in an elevated incidence of sister-chromatid exchange [14] and hSuv3 has been found to have several nuclear interaction partners involved in nuclear genome maintenance including BLM, WRN, RPA and FEN1 in human cells [12,14] and Sgs1, Ddc1 and Mec3 in yeast [15].

The Suv3 protein belongs to the SF2 superfamily of helicases [16]. It catalyzes the ATP-dependent separation of double stranded DNA and RNA substrates [11,12]. The hSuv3 unwinding activity is known to be strictly dependent on the presence of a divalent cation [11], however, the specific efficiency of different divalent cations is unknown. Stabilization of nucleotide binding by (RecA)-like helicase domains by way of coordination with a divalent cation is a common feature among most SF2 helicase family members [17]. Previously, various protein activities were shown to be differentially affected by different divalent cations. Interestingly, the mitochondrial polymerase gamma (Polγ) requires either Mg^{2+} or Mn^{2+} for *in vitro* activity, and while the biological role is currently unclear, Mn^{2+} supports reverse transcriptase activity of Polγ at the cost of fidelity [18,19]. Also, the helicase activity of the WRN helicase can be supported by Mn^{2+} or Ni^{2+} in addition to Mg^{2+}, while its exonuclease activity is stimulated in the presence of Zn^{2+} [20].

Suv3 is highly conserved across species but shares less than 25% sequence similarity to any known protein structure [16,21]. Based on sequence analysis and inspection of the crystal structure it was found that of the classical nine DEAD and DExD/H box helicase motifs (Q, I, Ia, Ib, II-VI), Suv3 lacks the Q-motif involved in ATP binding [21,22]. Normally, residues within the Q-motif interact specifically with atoms of the adenine group of ATP [22]. Instead, the hSuv3 protein was found to interact with the nucleotide via base-stacking between aromatic amino acids within a RecA domain with no specific interactions between protein and the adenine base atoms [21]. This may suggest that other nucleotides can be utilized by hSuv3 as well.

ATP is a major energy cofactor within the cell and is used by many different proteins in various cellular pathways [17]. Some helicases are restricted to the use of ATP, while others are more permissive allowing the use of other classic nucleotides e.g. UTP, GTP and CTP [23]. The mitochondrial helicase Twinkle is able to utilize all four ribonucleotides, with UTP resulting in the most efficient dsDNA unwinding activity [24,25]. In *T. brucei*, the mitochondrial degradosome activity was found to be stimulated *in organello* by increasing local UTP concentration. The ability of Suv3 to utilize energy cofactors other than ATP has not been addressed previously, although it may be important in regulating activity according to local or global changes in energy metabolism and nucleotide pools.

Here we examined the effect of inorganic cofactors on hSuv3 activity. Using an *in vitro* biochemical assay, we have analyzed the ATPase and helicase unwinding activity of hSuv3 in the presence of different divalent cations. Having identified the divalent cations that are able to support hSuv3 function, we next compared the efficiency of hSuv3 in the presence of the four major ribonucleotides, ATP, UTP, GTP and CTP, finding a divalent cation dependent difference in hSuv3 DNA unwinding efficiency between the ribonucleotides analyzed.

2. Material and methods

2.1. Recombinant proteins

Recombinant hSuv3 protein, hSuv3-(47–722)-WT and hSuv3-(47–722)-K213A helicase-dead mutant, were expressed in *E. coli* and purified to apparent homogeneity as described previously (Fig. S1) [12].

2.2. Oligonucleotide substrate

PAGE-purified oligonucleotides were used for preparation of substrates: D49, 5′-TTT GTT TGT TTG TTT GTT TGT TTG CCG ACG TGC CAG GCC GAC GCG TCC C-3′; D50-2, 5′-GGG ACG CGT CGG CCT GGC ACG TCG GCT TTG TTT GTT TGT TTG TTT GTT TT-3′. ^{32}P 5′-end labeling of the D50-2 oligonucleotide was performed as described previously [12]. In brief, D50-2 was incubated with [γ-^{32}P] ATP and T4 polynucleotide kinase at 37 °C. Labeling was terminated by adding EDTA to a final concentration of 10 mM. Unincorporated [γ-^{32}P] ATP was removed using a G-25 spin column. KCl was added to the eluate to a final concentration of 50 mM together with 1.5-fold excess of the D49 oligonucleotide and the two were annealed by initial incubation at 70 °C for 10 min, followed by slow-cooling of the reaction to room temperature.

2.3. Helicase activity assay

Helicase unwinding assessment was performed as described previously with some modifications [12]. Briefly, the recombinant hSuv3(47–722) protein (50 nM) was added to the reaction buffer containing 20 mM Tris–HCl, pH 7.4, 1 mM ATP if not otherwise indicated, 3 mM divalent cation if not otherwise specified, 1 mM DTT, 5% glycerol and 0.1 mg/ml BSA. The reactions were initiated by addition of the ^{32}P 5′-end-labeled substrate (0.5 nM), incubated at 37 °C for 30 min, and terminated by addition of helicase stop buffer to a final concentration of 10 mM Tris–HCl, pH 8.0, 10 mM EDTA, 10% glycerol, 0.3% SDS, 0.01% Bromophenol Blue and 5 nM unlabeled oligonucleotide. The products were resolved on a native 12% polyacrylamide gel and detected using a PhosphorImager followed by analysis using QuantityOne software.

2.4. ATPase activity assay

ATPase activity assay was conducted as described before, but with some modifications [26]. In short, reactions were initiated by adding recombinant hSuv3(47–722) protein (50 nM) to the reaction buffer containing 20 mM Tris–HCl, pH 7.4, 990 μM ATP, 10 μM γ-P^{32} ATP, 3 mM divalent cation, 1 mM DTT, 5% glycerol and 0.1 mg/ml BSA with or without 0.5 nM unlabeled DNA. Reactions were incubated at 37 °C for 30 min and stopped by adding EDTA to a final concentration of 50 mM. Samples were analyzed by thin layer chromatography in 0.75 M KH_2PO_4 and the signal was detected using a PhosphorImager followed by analysis using QuantityOne software.

2.5. DNA filter binding

DNA filter binding was performed as described by others with modifications [27]. Recombinant hSuv3(47–722) protein (25, 50 and 100 nM) was added to the reaction buffer containing 20 mM Tris–HCl, pH 7.4, 1–5 mM AMP-PCP, 3 mM divalent cation (as indicated), 1 mM DTT, 5% glycerol and 0.1 mg/ml BSA. The reactions were initiated by addition of the ^{32}P 5′-end-labelled single stranded D50-2 substrate (0.5 nM) and incubated at 37 °C for 30 min. Reactions were moved to ice and loaded directly on a double membrane platform with a nitrocellulose membrane on top of a nylon membrane binding the flow through DNA not bound by protein. The membranes were equilibrated in and washed with the binding-buffer: 50 mM Tris pH 7.5, 5% glycerol and 1 mM EDTA. The result was visualized using a PhosphorImager followed by analysis using QuantityOne software.