ACTA ABP BIOCHIMICA POLONICA

Vol. 64, No 1/2017
177–181
https://doi.org/10.18388/abp.2016_1419

Regular paper

Human SUV3 helicase regulates growth rate of the HeLa cells and can localize in the nucleoli

Maciej Szewczyk[1], Natalia Fedoryszak-Kuśka[3], Katarzyna Tkaczuk[1], Jurek Dobrucki[4], Agnieszka Waligórska[4] and Piotr P. Stępień[1,2,3]✉

[1]Institute of Genetics and Biotechnology, Faculty of Biology, Warsaw University, Warsaw, Poland; [2]Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland; [3]Centre for New Technologies, University of Warsaw, Warsaw, Poland; [4]Department of Cell Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

The human SUV3 helicase (SUV3, hSUV3, SUPV3L1) is a DNA/RNA unwinding enzyme belonging to the class of DexH-box helicases. It localizes predominantly in the mitochondria, where it forms an RNA-degrading complex called mitochondrial degradosome with exonuclease PNP (polynucleotide phosphorylase). Association of this complex with the polyA polymerase can modulate mitochondrial polyA tails. Silencing of the SUV3 gene was shown to inhibit the cell cycle and to induce apoptosis in human cell lines. However, since small amounts of the SUV3 helicase were found in the cell nuclei, it was not clear whether the observed phenotypes of SUV3 depletion were of mitochondrial or nuclear origin. In order to answer this question we have designed gene constructs able to inhibit the SUV3 activity exclusively in the cell nuclei. The results indicate that the observed growth rate impairment upon SUV3 depletion is due to its nuclear function(s). Unexpectedly, overexpression of the nuclear-targeted wild-type copies of the SUV3 gene resulted in a higher growth rate. In addition, we demonstrate that the SUV3 helicase can be found in the HeLa cell nucleoli, but it is not detectable in the DNA-repair foci. Our results indicate that the nucleolar-associated human SUV3 protein is an important factor in regulation of the cell cycle.

Key words: SUV3 helicase, SUPV3L1, dual targeting, nucleolus, mitochondria, cell cycle

Received: 02 September, 2016; revised: 13 January, 2017; accepted: 16 January, 2017; available on-line: 15 March, 2017

✉e-mail: stepien@ibb.waw.pl
Abbreviations: mt, mitochondrial; SUV3, Supressor of Var1

INTRODUCTION

In eukaryotic cells localization of a single gene product to more than one subcellular compartment is a common phenomenon, termed dual targeting, dual localization or dual distribution. Often, the amount of a given protein in the second subcellular compartment is very small or the protein resides in the second compartment only in a specific metabolic status of the cell. Such cases are difficult to detect by standard methods due to a strong signal from the major compartment and are described as an "eclipsed localization" (reviewed by Yogev & Pines, 2011). Dual localization provides a significant challenge in distinguishing which phenotype is related to which subcellular site of activity.

The SUV3 gene encoding an RNA helicase was discovered in the yeast *Saccharomyces cerevisiae* and the SUV3 protein was found to localize in mitochondria, where together with the DSS1 exoribonuclease, it forms an RNA-degrading complex called mtEXO or mitochondrial degradosome (Stepien *et al.*, 1992; Dziembowski *et al.*, 2003). Inactivation of the SUV3 gene resulted in a massive accumulation of mitochondrial RNA and in the absence of mitochondrial respiration. This prompted a hypothesis that the accumulated mtRNA is toxic for mitochondria (Margossian *et al.*, 1996). Indeed, by lowering the mitochondrial transcription rate, it was possible to rescue the respiratory phenotype of the SUV3 depletion in yeast cells (Rogowska *et al.*, 2006).

The SUV3 gene is present in purple bacteria and all eukaryotes (Dmochowska *et al.*, 1999). The human SUV3 helicase is encoded by a nuclear gene (SUV3, hSUV3, SUPV3L1) and is a DNA/RNA helicase belonging to the family of DExH-box helicases, able to unwind RNA, RNA-DNA or DNA duplexes (Minczuk *et al.*, 2002). The predominant subcellular localization of the SUV3 helicase is the mitochondrion, although small amounts have been detected in the cell nuclei (Szczesny *et al.,* 2007), which is in agreement with the nuclear localization sequence (NLS) present close to the C-terminus of this protein (Shu *et al.*, 2004). In human mitochondria, the SUV3 helicase forms an RNA-degrading complex with PNPase (polynucleotide phosphorylase), localized in the intra-mitochondrial granules (foci) which participate in the mtRNA decay (Szczesny *et* al., 2010; Borowski *et al.*, 2013). Recently, the SUV3-PNP-polyA polymerase complex has been implicated in modulation of the mitochondrial polyA tail lengths (Wang *et al.*, 2014) In addition, SUV3 haploinsufficiency in mice was found to induce mitochondrial genome instability leading to tumorigenesis and shortened lifespan (Chen *et al.*, 2013). Interestingly, mice with a conditional knock-out of the SUV3 gene developed premature aging, cachexia, sarcopenia and skin disease (Paul *et al.*, 2009).

Silencing of the SUV3 gene in human cell lines results in a significant accumulation of various classes of undegraded mitochondrial RNA (Szczesny *et al.,* 2010); in addition, cell cycle perturbations and apoptosis were observed (Szczesny *et al.*, 2007). This could be analogous to yeast, where a lack of mtRNA degradation resulted in a collapse of mitochondrial functions (Rogowska *et al.*, 2006). On the other hand, since a small fraction of the human SUV3 protein was found in the nucleus, it was not clear which phenotypes could result from the depletion of the nuclear fraction of the SUV3 protein and which from the mi-

tochondrial one. This was due to the fact that experiments reported so far were based on silencing of the whole SUV3 gene.

While mitochondrial functions of the SUV3 protein have been extensively investigated, much less is known about its putative role in the nucleus. The analysis of SUV3 interactors resulted in identification of the WRN and BLM helicases (Pereira *et al.*, 2007), FEN 1 flap endonuclease and replication protein A (Veno *et al.*, 2011). All of these proteins play important roles in chromatin maintenance and DNA repair, but no specific role for SUV3 in these complexes has been proposed.

This paper presents the first experimental approach to dissect the mitochondrially-related phenotypes of the SUV3 helicase depletion from the nuclear ones.

MATERIALS AND METHODS

Cell culture and cell manipulations. T-REx™-HeLa cells (Invitrogen) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (Gibco) at 37°C under 5% CO_2. The stable, inducible cell lines obtained in this work were cultured by using TET System Approved FBS (Clontech). For cell growth rate assessment 3.5×10^6 of the appropriate cells were plated, induced (or not) after 24 h and detached after the next 24 h. Every second day the cells were detached, counted and plated at the same density as before.

Development of stable transfected cell lines. We used DNA constructs created earlier by Szczesny et al. (2009): pRS68, pRS97, pRS98 and pRS100. These plasmids were based on the pcDNA5/FRT/TO vector (Invitrogen) backbone and encoded N-terminally truncated (ΔMTS, non-mitochondrial), full length wild-type (WT), full-length catalytically inactive (G207V) and N-terminally truncated, catalytically inactive (ΔMTS G207V) SUV3, respectively. The host cell-line was co-transfected using TransIT 2020 (Mirus) reagent with 0.4 μg of appropriate construct and 1.6 μg of pOG44 (Invitrogen). Twenty-four hours after transfection, cells were plated and subjected to selection by adding hygromycin B (200 μg/ml) (Invitrogen). Selective medium was replaced every 2–3 days. In all presented experiments, expression of the exogenous genes was induced with tetracycline (Sigma-Aldrich) at the concentration of 100 ng/ml.

Proliferation assay. A real-time cell proliferation assay was performed using the xCELLigence Real-Time Cell Analyzer DualPlate (RTCA DP)(ACEA Biosciences). This system uses measurement of electrical impedance, created by cells attached to the microelectrode (Irelan *et al.*, 2011). Cells were trypsinized, counted using an automated cell counter EVE (NanoEnTek) and plated at 5000 cells per well in DMEM (Gibco) supplemented with 10% fetal bovine serum (Gibco) and 100 ng/ml tetracycline (Sigma-Aldrich) in four replicates. Stable cell lines were grown under standard conditions (at 37°C under 5% CO_2). A unit-less parameter termed the Cell Index was derived and used to represent the cell number based on the measured relative change in electrical impedance that occurred in the presence and absence of cells in the wells (Xing *et al.*, 2005). The Cell Index was monitored every thirty minutes throughout 4 days of the experiment.

Immunofluorescence. 24 h prior the experiment cells were trypsinised, counted and plated at 50 000 cells per well of a 6-well plate (glass coverslip) in a medium supplemented with 100 ng/ml tetracycline. Then the cells were challenged with 5 uM camptothecin for 0, 3 or 6 h.

Cells were fixed with 4% formaldehyde, permeabilized with 0.5% Triton X-100 (Sigma-Aldrich) in PBS and blocked in 10% FBS in PBS. SUV3 was visualized using primary rabbit polyclonal antibodies described previously by Mińczuk and coworkers (2002), and secondary goat anti-rabbit antibodies coupled with Alexa555 (LifeTechnologies). To visualize phosphorylated histone H2AX we used Abcam mouse monoclonal antibodies (ab22551) and goat anti-mouse antibodies coupled with Alexa488 (LifeTechnologies). Coverslips were mounted with ProLong Gold Antifade Reagent (LifeTechnologies).

Images were acquired by laser scanning confocal microscope Leica TCS SP5 (Leica Microsystems GmbH, Wetzlar, Germany) with 63x plan apochromatic oil immersion objective lens (NA 1.4). The confocal pinhole size was set at 1 Airy unit. Laser line 488nm and 633nm were used for fluorescence excitation. Emission was collected in the range of 496–544nm and 650–735nm. Transmitted light images were also registered.

Western blotting. Wild-type HeLa cells were challenged with camptothecin as depicted above, detached and lysed in a cold RIPA buffer (Sigma-Aldrich). Protein concentration was determined by the Bradford method. 50 micrograms of each total protein extract were separated on 12.5% or 8% SDS-PAGE gels and transferred to PVDF membranes.

SUV3 and γH2AX were immunodetected with the same primary antibodies as used in IF while phosphorylated ATM kinase and β-actin, which was used as a loading control, were detected with CellSignalling mouse polyclonal antibodies (#4526 and #3700, respectively). Primary antibodies were detected with goat anti-rabbit or anti-mouse HRP-conjugated antibodies (Calbiochem) and visualized by enhanced chemiluminescence (BioRad).

RESULTS AND DISCUSSION

Silencing of the human SUV3 gene was previously achieved using three techniques: 1. by employing siRNA (Szczesny *et al.*, 2007), 2. by expression of a missense mutant lacking the helicase activity: complexes harboring such mutant are nonfunctional (Szczesny *et al.*, 2009; Borowski *et al.*, 2012) and 3. by constructing mouse models harboring a deletion or a conditional KO of the SUV3 gene (Pereira *et al.*, 2007; Paul *et al.*, 2009).

In all of the three approaches both, mitochondrial and nuclear functions of the SUV3 helicase were affected, so the resulting cellular or organismal phenotypes could be not be attributed to the SUV3 deficiency in a particular subcellular compartment.

In order to address the question whether the growth rate inhibition depends on the nuclear or mitochondrial SUV3 functions, we constructed HeLa cell lines with inducible gene constructs: two of them were missense mutants in the SUV3 helicase domain with the substitution of glycine for valine at position 207 of the Walker A helicase motif within the SUV3 open reading frame. This mutation completely abolishes the helicase activity (Minczuk *et al.*, 2002). In addition, we deleted a 46 amino acid N-terminal mitochondrial targeting sequence (MTS) (Delta46aa; SUV3:G207V), thus impairing the transport of the protein into mitochondria. In addition, we also constructed cell lines containing a wild-type helicase, with MTS (wtSUV3) and

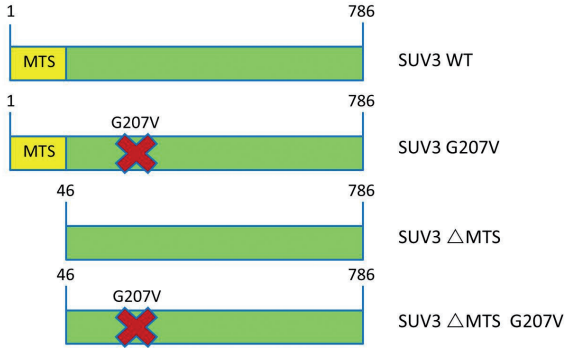

Figure 1. Schematic representation of the gene constructs used.
MTS, mitochondrial targeting sequence of 46 amino acid; G207V, the substitution of valine instead of glycine at the position 207 of the Suv3p ORF, inactivating the Walker A helicase motif.

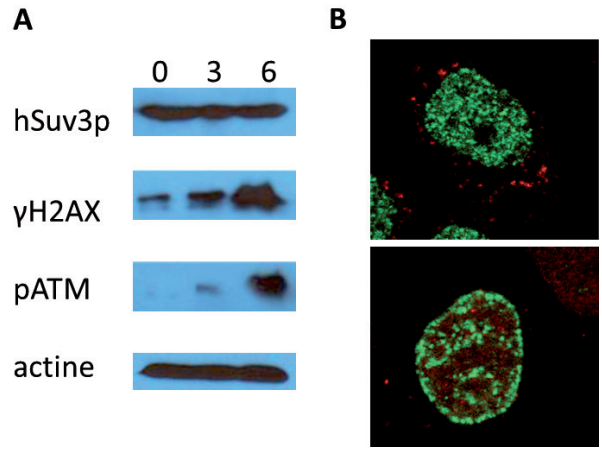

Figure 3. SUV3 is not directly involved in the double-strand DNA breaks repair.
(**A**) Western blot showing induction of the ATM kinase and H2AX histone phosphorylation after the HeLa cells were challenged with 5 μM camptothecin for the times indicated. SUV3 is not induced under these conditions. (**B**) SUV3 (red colour) does not co-localize with the DNA repair foci (green, visualized by γH2AX immunodetection). Top: wild-type HeLa cells, bottom: HeLa cells overexpressing ΔMTS SUV3.

without MTS (Delta46aa; SUV3:G207V). All constructs are schematically shown in Fig. 1. The constructs were stably incorporated into nuclear genomes of the human HeLa cell line using the Flp-In technique. Addition of tetracycline to the medium resulted in induction of the introduced genes. A similar approach has been previously developed in our lab (Szczesny *et al.*, 2010) in human 293 cells, but no growth studies were performed. The growth of the cell lines was measured in the Real-Time Cell Analyzer Dual Plate (RTCA DP, xCELLigence device), where cellular growth was continuously monitored and calculated during 65 hours after gene induction (Fig. 2).

The results presented here indicate that both strains carrying mutations in the helicase domain, either with or without the mitochondrial localization signal (MTS), show a strong inhibition of growth rate. Our previous studies demonstrated that the mutant protein with no MTS does not localize to mitochondria and exclusively localizes to the nucleus (Szczesny *et al.*, 2007); therefore the growth inhibition observed here is not due to the disturbances in mitochondrial RNA metabolism, but is the consequence of the perturbation of a yet unknown nuclear function of the SUV3 protein. Our result is also in line with the previous observations that the down regulation of the SUV3 gene induces caspase- and AIF- dependent apoptotic pathways (Szczesny *et al.*, 2007).

The growth curves presented in Fig. 2 show the unexpected second phenotype associated with the nuclear function of the SUV3 protein: overexpression of the nuclear-localized wild-type helicase results in a higher growth rate. This effect is stronger when the wild-type allele is driven to the cell nuclei by the absence of a mitochondrial targeting sequence. Both constructs, with or without the MTS, drive the growth rate above the control level. Interestingly, similar effects were observed in plants, where overexpression of the full-length wild-type plant SUV3 gene resulted in improved stress resistance in rice, delayed leaf senescence-associated events and resulted in 3.5-fold increase in the

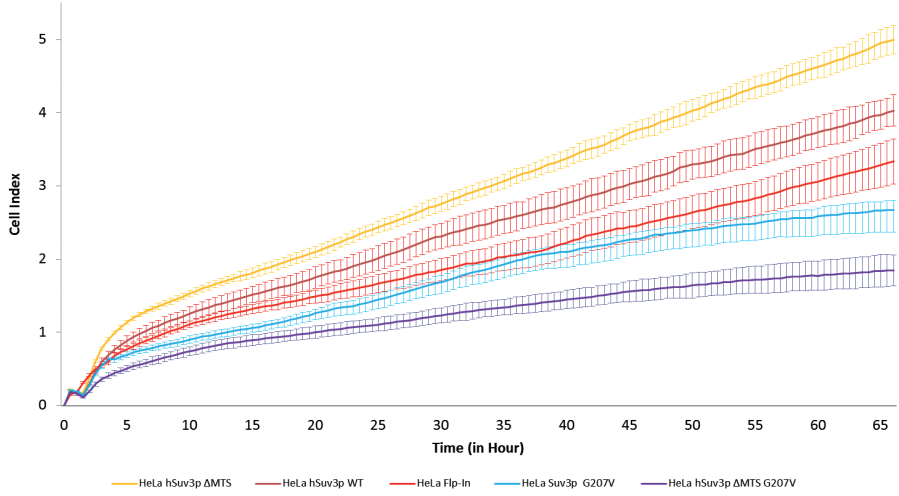

Figure 2. Growth curves of HeLa cells harboring different SUV3 constructs.
Measurements were taken in an automated cell counter. Proliferation of HeLa mutated cells was analyzed by the xCELLigence system, operating on the basis of electrical resistance measurement. Resistance was measured every 30 minutes for 65 hours. The final cell index values show the difference between the resistance generated by the cells at each time point and the resistance of the medium without cells. The graph indicates proliferation index at each time point and SD for each measurement.

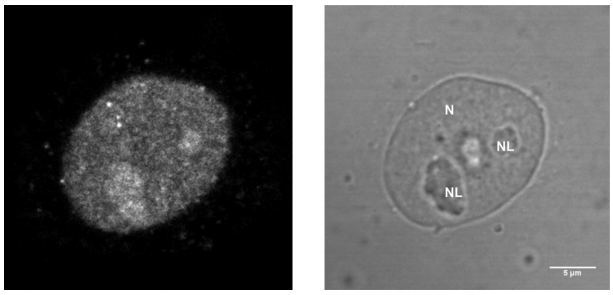

Figure 4. Localization of ΔMTS SUV3 in the nucleoli.
(**Left**) SUV3 visualized in the whole nucleus by immunofluorescence, (**Right**) a transmitted light image of the same cell (N, nucleus; NL, nucleoli). Overexpressed ΔMTS SUV3 is localized almost exclusively in the cell nucleus. Intranuclear regions of the increased SUV3 fluorescence signal intensity co-localize with nucleoli.

telomere length (Sahoo *et al*., 2014; Sahoo *et al*., 2015; Macovei *et al*., 2016).

Human SUV3 protein was found to interact with several nuclear proteins involved in chromatin stability. Therefore we decided to assess if the SUV3 protein co-localizes with the DNA repair foci which form upon DNA damage. Such foci can be visualized by immunofluorescence of 53BP1 and γH2AX proteins. We challenged the cells using camptothecin in order to induce double strand breaks and checked whether signal from the SUV3 antibodies co-localizes with the repair foci. While we could detect the induction of both, the 53BP1 and γH2AX proteins (Fig. 3, panel A) on a Western blot, and the DNA repair foci visualized by immunofluorescence, the SUV3 helicase signal did not co-localize with the foci (Fig. 3 panel B). Thus we suggest that the SUV3 helicase does not participate in the repair of the DNA double strand breaks.

Finally we investigated the sub-nuclear localization of the SUV3 protein. Our previous data indicate that the nuclear level of the SUV3 protein is rather low and it depends on the cell line: signal in the HeLa cells is much stronger than in the 293 cells. Therefore, we induced our SUV3:DELTA46 construct in the HeLa cells, reasoning that the lack of mitochondrial localization signal would drive the overexpressed protein to the cell nuclei and thus increase the signal to noise ratio. Indeed, by using anti-SUV3 antibodies we were able to detect the intranuclear foci of the SUV3 protein by immunofluorescence, which co-localize with nucleoli appearing in the visible light (Fig. 4). Recent data suggest that nucleoli exert control over cell cycle progression in both, forward and reverse ways (reviewed by Tsai and Pederson, 2016). Thus, it is tempting to speculate that the SUV3 helicase is a part of this regulatory circuit, but clearly more research is needed.

In summary, we demonstrate here the importance of nuclear localization of the SUV3 protein for regulation of the cell cycle in HeLa cells. To the best of our knowledge, this is the first report dissecting the mitochondrial phenotypes of SUV3 from the nuclear ones. The results presented here are in agreement with previously detected interactions of the human SUV3 protein with nuclear chromatin maintenance factors, yet it seems that SUV3 does not participate in the DNA repair. Our study was conducted on cell lines, but it calls for re-visiting of the data obtained with conditional KO of the SUV3 gene in mice: SUV3-associated phenotypes including cancer (Chen *et al*., 2013) and premature aging (Paul *et al*., 2009) could possibly be due to the involvement of the SUV3 helicase in nucleolar functions.

Acknowledgements

This work was supported by the National Science Centre, Poland, by the grants No 2013/11/B/NZ1/0089 and 2013/11/B/NZ3/00189.

M.S. and N.F-K contributed equally to this work. We thank Dr. Roman Szczęsny for his kind help with preparing the DNA constructs.

REFERENCES

Borowski LS, Dziembowski A, Hejnowicz MS, Stepien PP, Szczesny RJ (2013) Human mitochondrial RNA decay mediated by PNPasehSuv3 complex takes place in distinct foci. *Nucleic Acids Res* **41**: 1223–1240. DOI: 10.1093/nar/gks1130

Chen PL, Chen CF, Chen Y, Guo XE, Huang CK, Shew JY, Reddick RL, Wallace DC, Lee WH (2013) Mitochondrial genome instability resulting from SUV3 haploinsufficiency leads to tumorigenesis and shortened lifespan. *Oncogene* **32**: 1193–1201. DOI: 10.1038/onc.2012.120

Dmochowska A, Kalita K, Krawczyk M, Golik P, Mroczek K, Lazowska J, Stepień PP, Bartnik E (1999) A human putative Suv3-like RNA helicase is conserved between *Rhodobacter* and all eukaryotes. *Acta Biochim Pol* **46**: 155–162

Dziembowski A, Piwowarski J, Hoser R, Minczuk M, Dmochowska A, Siep M, van der Spek H, Grivell L, Stepien PP (2003) The yeast mitochondrial degradosome. Its composition, interplay between RNA helicase and RNase activities and the role in mitochondrial RNA metabolism. *J Biol Chem* **278**: 1603–1611. DOI: 10.1074/jbc.M208287200

Irelan JT, Wu MJ, Morgan J, Ke N, Xi B, Wang X, Xu X, Abassi YA (2011) Rapid and quantitative assessment of cell quality, identity, and functionality for cell-based assays using real-time cellular analysis. *J Biomol Screen.* **16**: 313–322. DOI: 10.1177/1087057110397359

Macovei A, Sahoo RK, Fae M, Balestrazzi A, Carbonera D, Tuteja N, (2016) Overexpression of PDH45 or SUV3 helicases in rice leads to delayed leaf senescence-associated events. *Protoplasma* published online. DOI 10.1007/s00709-016-1017-4

Margossian SP, Li H, Zassenhaus HP, Butow RA (1996) The DExH box protein Suv3p is a component of a yeast mitochondrial 3'-to-5' exoribonuclease that suppresses group I intron toxicity. *Cell* **84**: 199–209

Minczuk M, Piwowarski J, Papworth MA, Awiszus K, Schalinski S, Dziembowski A, Dmochowska A, Bartnik E, Tokatlidis K, Stepien PP, Borowski P (2002) Localisation of the human SUV3 helicase in the mitochondrial matrix and its preferential unwinding of dsDNA. *Nucleic Acids Res* **30**: 5074–5086.

Paul E, Cronan R, Weston PJ, Boekelheide K, Sedivy JM, Lee SY, Wiest DL, Resnick MB, Klysik JE (2009) Disruption of Supv3L1 damages the skin and causes sarcopenia, loss of fat, and death. *Mamm Genome* **20**: 92–108. DOI: 10.1007/s00335-008-9168-z

Pereira M, Mason P, Szczesny RJ, Maddukuri L, Dziwura S, Jedrzejczak R, Paul E, Wojcik A, Dybczynska L, Tudek B, Bartnik E, Klysik J, Bohr VA, Stepien PP (2007) Interaction of human SUV3 RNA/DNA helicase with BLM helicase; loss of the SUV3 gene results in mouse embryonic lethality. *Mech Ageing Dev* **128**: 609–617. DOI: 10.1016/j.mad.2007.09.001

Rogowska AT, Puchta O, Czarnecka AM, Kaniak A, Stepien PP, Golik P (2006) Balance between transcription and RNA degradation is vital for *Saccharomyces cerevisiae* mitochondria: reduced transcription rescues the phenotype of deficient RNA degradation. *Mol Biol Cell.* **17**: 1184–1193. DOI: 10.1091/mbc.E05-08-0796

Sahoo RK, Ansari MW, Tuteja R, Tuteja N (2014) OsSUV3 transgenic rice maintains higher endogenous levels of plant hormones that mitigates adverse effects of salinity and sustains crop productivity. *Rice (N Y).* **7**: 17. DOI: 10.1186/s12284-014-0017-2

Sahoo RK, Ansari MW, Tuteja R, Tuteja N (2015) Salt tolerant SUV3 overexpressing transgenic rice plants conserve physicochemical properties and microbial communities of rhizosphere. *Chemosphere* **119**: 1040–1047. DOI: 10.1016/j.chemosphere.2014.08.011

Shu Z, Vijayakumar S, Chen CF, Chen PL, Lee WH (2004) Purified human SUV3p exhibits multiple-substrate unwinding activity upon conformational change. *Biochemistry* **43**: 4781–4790. DOI: 10.1021/bi0356449

Stepien PP, Margossian SP, Landsman D, Butow RA (1992) The yeast nuclear gene suv3 affecting mitochondrial post-transcriptional pro-

cesses encodes a putative ATP-dependent RNA helicase. *Proc Natl Acad Sci* **89**: 6813–6817

Szczesny RJ, Obriot H, Paczkowska A, Jedrzejczak R, Dmochowska A, Bartnik E, Formstecher P, Polakowska R, Stepien PP (2007) Down-regulation of human RNA/DNA helicase SUV3 induces apoptosis by a caspase- and AIF-dependent pathway. *Biol Cell* **99**: 323–332. DOI: 10.1042/BC20060108

Szczesny RJ, Borowski LS, Brzezniak LK, Dmochowska A, Gewartowski K, Bartnik E, Stepien PP (2010) Human mitochondrial RNA turnover caught in flagranti: involvement of SUV3 helicase in RNA surveillance. *Nucleic Acids Res* **38**: 279–298. DOI: 10.1093/nar/gkp903

Tsai RYL, Pederson T (2016) Connecting the nucleolus to the cell cycle and human disease. *FASEB J* **28**: 3290–3296. DOI: 10.1096/fj.14-254680

Veno ST, Kulikowicz T, Pestana C, Stepien PP, Stevnsner T, Bohr VA (2011) The human Suv3 helicase interacts with replication protein A and flap endonuclease 1 in the nucleus. *Biochem J* **440**: 293–300. DOI: 10.1042/BJ20100991

Wang DD, Guo XE, Modrek AS, Chen CF, Chen PL, Lee WH (2014) Helicase SUV3, polynucleotide phosphorylase, and mitochondrial polyadenylation polymerase form a transient complex to modulate mitochondrial mRNA polyadenylated tail lengths in response to energetic changes. *J Biol Chem* **289**: 16727–16735. DOI: 10.1074/jbc.M113.536540

Xing JZ, Zhu L, Jackson JA, Gabos S, Sun XJ, Wang XB, Xu X (2005) Dynamic monitoring of cytotoxicity on microelectronic sensors. *Chem Res Toxicol* **18**: 154–161. DOI: 10.1021/tx049721s

Yogev O, Pines O (2011) Dual targeting of mitochondrial proteins: Mechanism, regulation and function. *Biochim Biophys Acta* **1808**: 1012–1020. DOI: 10.1016/j.bbamem.2010.07.004