OnLine Journal of Biological Sciences

Original Research Paper

The Silencing of *Septin 1* via Synthetic siRNAs in *Schizosaccharomyces pombe*

Semih Ekimler, Merve Yilmazer and Ercan Arican

Department of Molecular Biology and Genetics, Faculty of Science, Istanbul University, Istanbul, Turkey

Article history
Received: 07-02-2017
Revised: 22-03-2017
Accepted: 30-03-2017

Corresponding Author:
Ercan Arican
Department of Molecular Biology and Genetics, Faculty of Science, Istanbul University, Istanbul, Turkey
Ph: +902124555700
Email: earican@istanbul.edu.tr

Abstract: *Septins* are evolutionary conserved GTP-binding proteins and form heterooligomers in cells to interact with cytoskeleton. The main roles of *septins* in cell are cytokines, membrane interactions, vesicle trafficking and microtubule and actin organization. Non-coding RNAs are key players of gene expression regulation. Small-interfering RNAs (siRNAs) belong to non-coding RNAs, are characterized by sequence complementarity to their target mRNAs. siRNAs silence their targets via mRNA degradation or inhibition of translation. In *S. pombe* siRNAs can also propagate heterochromatin to silence genes located in centromeric regions. In this study, the aim was understanding the results of extrinsically induced siRNA silencing in *S. pombe* by using synthetic siRNAs specific to *Septin 1* gene. siRNAs were introduced to cells by lipid-based vesicles and the alterations in *Septin 1* expressions were monitored with qPCR. *Septin 1* expression was reduced dramatically via direct siRNA introduction to cells. The interactions between cell morphology and the reduced level of *Septin 1* were observed after accomplishing the silencing of *Septin 1* gene. In this study, it is shown that the morphology of the cells is affected by the reduced levels of *Septin 1* expression and the cells form long and chain-like structures. This study constitutes an important example in understanding the results of inducing siRNA silencing extrinsically in *S. pombe*.

Keywords: RNAi, Fission Yeast, *Septin*, Cell Division

Introduction

Septins belong to guanine nucleotide-binding proteins that polymerize into filaments and have been recognized as important members of the cytoskeleton in recent years. They were first discovered in *Saccharomyces cerevisiae* through screening of gene mutations in cell-cycle progression, of which mutants caused morphological changes and disruption of cell division (Hartwell, 1971). Electron and fluorescence microscopy results revealed that *septins* localize in bud-neck separation as a ring and regulate cell division (Haarer and Pringle, 1987). Subsequently, *septins* were further characterized and were found conserved in all animals and fungi but not in plants (Kinoshita, 2003). Seven *septin* genes have been identified both in *S. cerevisiae* and *Schizosaccharomyces pombe* while there are 12 loci for *septins* in human genome (Kinoshita, 2003; Russell and Hall, 2011).

Inside the cell, *septins* form hetero-oligomeric filaments that are composed of repetitive tetrameric, hexameric and octameric structures (Spiliotis *et al*., 2008). They also generate rings and cage-like structures. These formations are functionally active in the cell and act as molecular scaffolds for recruiting other proteins and as diffusion barriers for cell compartmentalization (Mostowy and Cossart, 2012). *Septins* have been observed to associate with act in (Kinoshita *et al*., 2002), microtubules (Sellin *et al*., 2011) and phospholipid membranes (Bertin *et al*., 2010). These associations define the forms of high-order structures.

The main role of these assemblies is to act as sub cellular scaffolds to mediate the accumulation of proteins involved during cytokines at the division site (Weems *et al*., 2014). They also play role in cell shape (Mostowy *et al*., 2011), cell polarity (Sirajuddin *et al*., 2007), cell movement (Gilden *et al*., 2012) and vesicle trafficking (Vega and Hsu, 2003).

The expression of *septins* is highly regulated in mammalian cells and they play critical roles in very diverse cellular functions, depending on their tissue-specific expressions and interacting molecules. Mutations and expression changes in *septins* have been associated with many cancer types and

neurodegenerative diseases. Understanding the functional roles of *septins* is important for the diagnosis, prognosis and possible therapies for septin-related diseases (Peterson and Petty, 2010).

Yeasts have been used as model organisms in investigating cellular processes such as cell division, DNA replication, metabolism, protein folding and intracellular transport (Fields and Johnston, 2005). *S. cerevisiae* has been the pioneering yeast as the first eukaryotic organism of which the genome was sequenced (Goffeau *et al*., 1996). Among the other yeasts, especially *S. pombe* has started to contribute later to the understanding of the molecular basis of processes that are common in higher eukaryotes (Bolotin-Fukuhara *et al*., 2010). Even though *S. cerevisiae* has the leading role for genetic research, *S. pombe* has a feature that makes it more interesting. During evolution, *S. pombe* kept the RNA interference (RNAi) mechanism. Its genome encodes genes required for RNAi machinery and small non-coding RNAs, which regulate gene expression via post-transcriptional gene silencing and heterochromatin propagation (Reyes-Turcu and Grewal, 2012).

RNAi is an evolutionarily conserved mechanism for gene regulation in fungi, plants and animals. It uses non-coding, double-stranded RNAs for targeting mRNAs, depending on sequence homology and for silencing genes via degrading mRNAs or dispersing heterochromatin (Fire *et al*., 1998). Based on the biogenesis and functions of these small non-coding RNAs, they are classified mainly in four groups: Short interfering RNAs (siRNAs), microRNAs (miRNAs), piwi-interacting RNAs (piRNAs) and long intervening non-coding RNAs (lincRNAs) (Ghildiyal and Zamore, 2009). siRNAs are derived from distinct exogenous sources such as viral RNAs, transposons and transgenes or endogenous sources such as repetitive sequences at centromeric and telomeric regions, convergent transcripts, sense-antisense pairs, gene/pseudogene duplexes and any RNAs that can form hairpin structure (Carthew and Sontheimer, 2009). Once they are in the cytoplasm, they are cleaved by Dicer and become mature to interact with Argonaute proteins, after which they are functionally active in RNA-Induced Silencing Complex (RISC). siRNAs regulate gene expression by base-pairing at the 3′-Untranslated Region (3′-UTR) of their target mRNAs and based on the level of sequence compatibility, they silence the expression of genes either via mRNA degradation or translational repression at post-transcriptional level (Filipowicz *et al*., 2005).

Due to the huge potential of gene regulation via non-coding RNAs, RNAi mechanism has been recognized as a new therapy strategy and these RNAi-based strategies have increased the possibility of curing fatal disorders such as neurodegenerative diseases, cancer, inflammatory diseases and viral infections (Chen *et al*., 2013). In order to efficiently deliver siRNAs into the cells, two main strategies have been designed. First one involves chemical modifications of siRNAs based on their charge, size, shape, base composition, surface chemistry and targeting motifs. The second is the exogenous compounds that mediate the delivery and they include viral vectors, plasmids, aptamers, liposomes, nanoparticles, polymers and dendrimers (Conde *et al*., 2015). Even though the most promising strategy is to use nanoparticles for siRNA delivery, lipid-based polymers have been used more widely in research (Kanasty *et al*., 2013).

In this study we aimed to silence *septin1* (*spn1*) gene of *S. pombe* with synthetic siRNAs. Three siRNAs were designed from the 3′-UTR, exonic region and 5′-UTR of *spn1* gene. A commercial transfection kit was used for siRNA delivery to the cell and following the siRNA transfection, qPCR analysis was carried out for the changes in the expression levels. Then the cells were transformed with a plasmid containing GFP-α-tubulin and following the siRNA transfection, changes in cell morphology were analysed by fluorescence microscopy. This study plays an important role in transcriptional gene silencing via siRNAs in *S. pombe* and also gives preliminary insight for synthetic siRNA delivery to *S. pombe* cells which has not been presented before.

Materials and Methods

Strains and Growth Conditions

S. pombe972h- wild-type strains and leu2h+ mutant strains were grown as recommended by Gutz *et al*. (1974) in standard rich media (YEL) and Minimal (EMM) media containing additional leucine (50 mg L^{-1}) respectively.

Primers and siRNA Design for spn1 Gene

The sequence of *spn1* gene was obtained from NCBI database and primers were selected based on the suggestions of NCBI Pick Primer software (Table 1). Three different siRNAs were designed by siRNA Wizard v3.1 software, provided by Invivogen. During siRNA design, siRNAs were selected from the 3′-UTR, 5′-UTR and exonic region of *spn1* gene (Table 2). Selected siRNAs were produced commercially by Dharmacon ON-TARGET plus (Thermo Scientific). Before using they were dissolved in 5X siRNA Buffer (Thermo Scientific) as including 20 nmol siRNA in each solution.

siRNA Silencing of spn1 Gene

siRNA delivery to cells was performed by cationic liposomes using Dharma FECT siRNA Transfection Reagent (Thermo Scientific). First, siRNAs were diluted in 5X siRNA Buffer (Thermo Scientific) to 5 nmol and 50 μL of this solution was mixed with 750 μL YEL

medium. Then, another 25 µL Dharma FECT siRNA Transfection Reagent without siRNA was mixed with 725 µL YEL medium. Both solutions were incubated 5 min at room temperature, then mixed and incubated on a shaker at room temperature for 20 min. After incubation, the mixture was added to 10 mL cell solution that includes 10^6 cells/ml. The cells were incubated in a shaker at 30°C for 4 h for siRNA silencing.

RNA Isolation and cDNA Synthesis

Following the silencing, total RNA isolation was performed using Gene JET RNA Purification Kit, according to manufacturer's instructions. Prior to using the kit, the cells were mechanically homogenized by vigorous shaking using glass beads and PBS. Subsequently isolated RNAs from samples were converted to cDNA by Revert Aid First Strand cDNA Synthesis Kit, according to manufacturer's instructions.

Gene Expression Analysis with Real-Time PCR

The changes in mRNA levels of *spn1* gene following siRNA silencing were investigated by Real-Time PCR using *S. pombe* actin primers as a housekeeping gene (Table 3) and SYBR Green I for fluorescent marker. The reaction was performed in Light Cycler 480 instrument (Roche). The *spn1* primers and cDNAs from samples were added to Maxima SYBR Green qPCR Master Mix (Thermo Scientific), according to manufacturer's instructions. Three biological and three technical replicas were performed for each samples.

Table 1. *spn1* primers for qPCR

Primers	G+C content (%)	T_m (°C)	Sequence (5′→3′)
Primer-F	55	60.04	GGGGTTCCGACGTTGATTCT
Primer-R	50	59.97	AAAATCGCCAAAACCGGGTG

Table 2. siRNAs for *spn1* silencing

siRNA	Sequence (5′→3′)	Starting position	G+C content (%)
1	GCGTCAGTTGAACGGATACGT	146	52.38
2	ACCCACCGACCCAGAAATCTT	301	52.38
3	GAAGAGGAGCGTGCCTTACAC	1098	57.14

Table 3. *actin* primers for qPCR

Primers	G+C Content (%)	T_m (°C)	Sequence (5′-3′)
Primer - F	50	56.1	AGATTCTCATGGAGCGTGGT
Primer - R	45	54.2	TCAAAGTCCAAAGCGACGTA

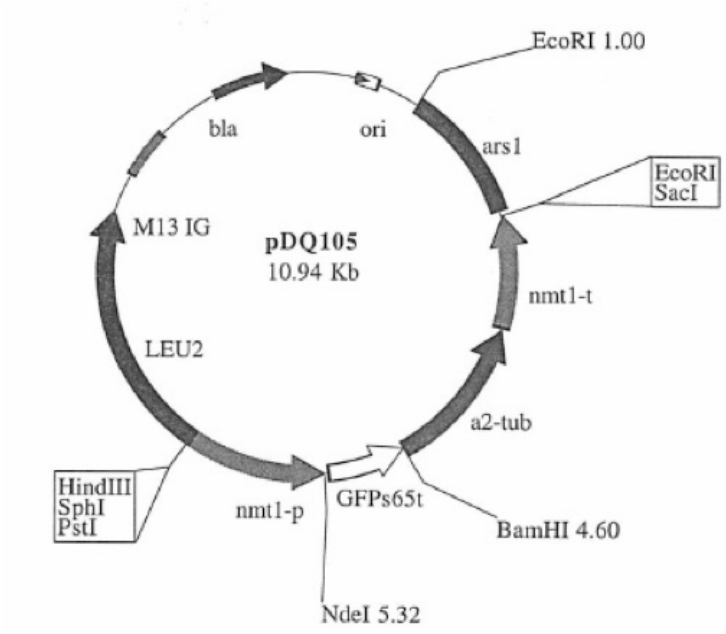

Fig. 1. pDQ105 plasmid for *S. pombe* transformation (ori: Origin of Replication, LEU2: Leucine 2 gene in yeast for selection, nmt1-p and nmt1-t: Thiamine promoter for yeast, GFPs65t: GFP gene, a2-tub: a2 tubulin gene)

Cell Growth Analysis after siRNA Silencing

Following the siRNA silencing described above, the growth of cells were measured spectrophotometrically. The optical density of cells was measured in every 2 h for 36 h at 600 nm wavelength. Three biological and three technical replicas were performed for each samples.

Plasmid Transformation to S. Pombe leu2h+ Cells

The pDQ105 plasmid was designed by Dr. Da-Qiao Ding and was acquired from National Bioresource Project, Yeast Genetic Resource Center Japan (YGRC/NBRP). The plasmid includes coding sequence of GFP-S65T adjacent to α2-tubulin and nmt1 promoter for the expression (Fig. 1). The transformant colonies were selected by the ampicillin resistance gene in *E. coli* and by *leucine 2* gene in *S. pombe* leu2h+ cells. The plasmid was transformed to competent *E. coli* DH5α cells via heat-shock method and plasmids were isolated from *E. coli* colonies on LBA + ampicillin media using ZyppyTM Plasmid Miniprep Kit (Zymo Research). Then plasmids were transformed to *S. pombe* cells using Lithium Acetate Method (Moreno *et al*., 1991) and the colonies were grown on EMM + leucine media.

Fluorescent Microscopy Analysis of spn1 Silencing

Transformant *S. pombe* leu2h+ cells were subjected to siRNA silencing by siRNA 3 as described above and the effects of silencing on phenotype have been analysed using Olympus BX53F fluorescent microscope. The cells were collected in the middle of the logarithmic phase and fixed by alcohol, according to Forsburg and Rhind (2006) method. In order to acquire fluorescent signals from the cells the U-FBW filter was used at 489 nm excitation wavelength and 509 nm emission wavelength, in 30 ms exposure time.

Results

The Expression Levels of spn1 were Reduced by Synthetic siRNAs

Three siRNAs from the 5′-UTR, exonic region and 3′-UTR of *spn1* gene were transferred to *S. pombe* 972h- cells separately, as combinations of two siRNAs and three of them together into the cells via cationic liposomes. Real-Time PCR analysis that compares relative *spn1* mRNA levels to *actin* mRNA levels showed that siRNA 3 which is complementary to the 3′-UTR of *spn1* reduced the expression of *spn1* the most. In addition to that, siRNA 2 which is complementary to the exonic region of the gene also reduced the expression of *spn1*. The combinations of siRNAs that include siRNA 3 had more silencing effect than other combinations (Fig. 2).

Cell Growth was Stalled after siRNA Silencing

In order to observe the effects of siRNA silencing on cell growth, the cells that were subjected to siRNA silencing with different siRNAs were incubated and their optical density was measured on 600 nm every 2 h. Based on the measurements, siRNA 3 caused a very long delay before entering the logarithmic phase compared to the effects of other siRNAs and also the optic density could not reach to the same level as the control group, indicating that cells could not grow or divide properly. Application of siRNA 1, combinations of two siRNAs and three siRNAs together showed moderate delay compared to control cells, however siRNA 2 showed no significant effect neither on entering logarithmic phase nor on cell number (Fig. 3).

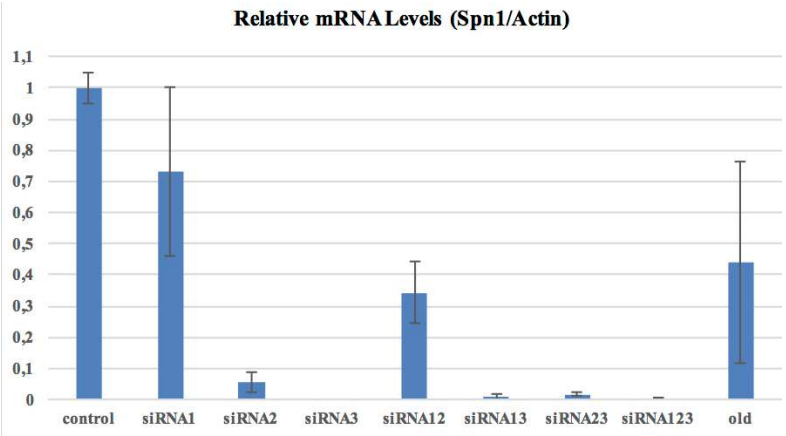

Fig. 2. qPCR results after siRNA silencing (control: Cells without siRNA applications, siRNA applications were numbered by used siRNA in each sample respectively, old: Cells from a culture that was incubated more than 36 h)

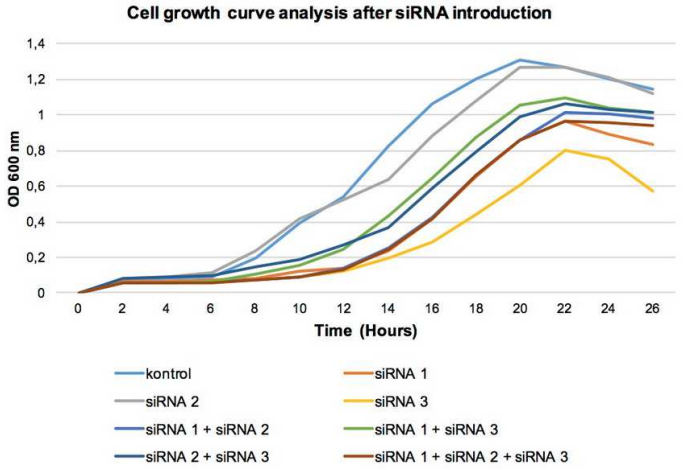

Fig. 3. Cell growth curve analysis after siRNA introduction (control: Cells without siRNA applications, siRNA applications were numbered by used siRNA in each sample respectively, old: Cells from a culture that was incubated more than 36 h)

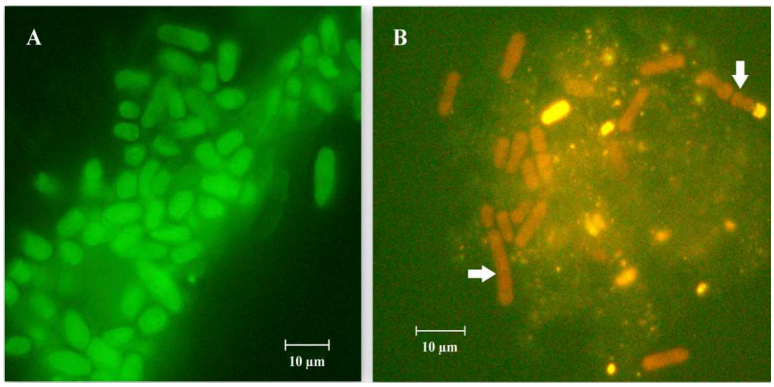

Fig. 4. Morphological analysis for changes in cell shape after siRNA silencing (A: Cells subjected to siRNA silencing, B: Control cells without siRNA silencing, Arrows: elongated and chain-like cells)

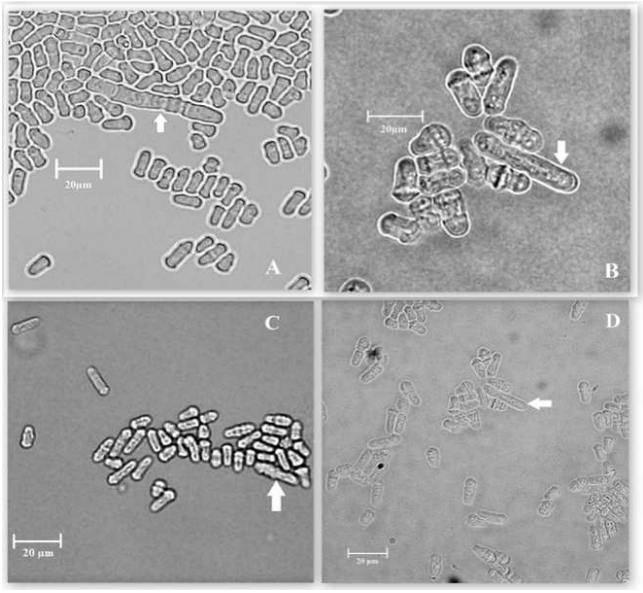

Fig. 5. Morphological Changes Following siRNA Silencing (A, B, C, D: Different samples from cells subjected to siRNA silencing, Arrows: Elongated and chain-like cells)

Table 4. Number of morphologically changed cells and their ratios to normal cells

Samples	Total cell count	Number of chain-shapedcells	Ratio
1	325	35	%10,7
2	89	14	%15,7
3	53	4	%7,5
4	107	16	%14,9

siRNA Silencing Caused Morphological Changes in Cell Shape

Based on acquired data from Real-Time PCR and cell growth analysis results, siRNA 3 that is complementary to 3′-UTR of *spn1* gene, was selected for further analysis of silencing on cell shape. The *S. pombe* leu2h+ cells were first transformed with pDQ105 plasmid and then siRNA 3 was transferred into cells. After incubation of 4 h with siRNA 3, the morphology of cells was changed to elongated, chain-like and multinucleated cells. The changes in the morphology were observed under fluorescent microscope (Fig. 4 and 5). Additionally, the number of morphologically elongated cells was counted under light microscope and the ratio of long cells was found 12% (Table 4).

Discussion

In this study, it was aimed to silence the expression of *spn1* gene in *S. pombe* via synthetic siRNAs. *Septins* were first identified in *S. cerevisiae* and associated with cell division. They are guanine nucleotide-binding proteins and form filaments in the cell (Pham *et al*., 2014). Septin filaments have a central role in cytokinesis, vesicular transport, cilia formation, phagocytosis, exocytosis, cell polarity, membrane dynamics, chromosome alignment and segregation, apoptosis and response to DNA damage (Weems *et al*., 2014). The expression of *septins* in the cell is regulated strictly both in a temporal and spatial manner and the changes in the expression lead to development of cancer, neurodegenerative diseases and infections. Due to their very crucial part in cellular processes it is very important to understand the molecular aspects of septin structure, function and interactions (Peterson and Petty, 2010).

In *S. pombe*, seven *septin* genes were identified and in this study *Septin1* was selected to be investigated due to its central role in heterooligomeric formation of septin complexes (An *et al*., 2004) and its homology with *Septin 6* in classification of *septins* in all organisms (Weirich *et al*., 2008). Human septin 6 is particularly important in neurodegenerative diseases due to its involvement in neuron migration, formation of aksons and dendrites and synaptic activity (Hu *et al*., 2012).

In recent years, siRNA-mediated gene silencing studies have been increasingly focusing in characterization of gene functions and RNAi-based methods have become a very important tool for developing new therapeutic strategies for cancer and neurodegenerative diseases. RNAi-mediated control of gene expression is a conserved process in almost all organisms. However, among the model organisms, *S. cerevisiae* does not have this feature while in *S. pombe*, the functional molecular such as Dicer, Ago and RdRP are coded as single copies in the genome and this makes *S. pombe* a more important model organism for RNAi studies (Dang *et al*., 2011).

RNAi-based gene silencing strategies are carried out in three ways: (i) by hairpin-structure RNA transcription from a transgene, (ii) by convergent transcription of RNA from genes of interest and (iii) by direct introduction of double-stranded RNAs into cells. The last method has been used very frequently in mammalian cells but it is not very common in yeast (Li *et al*., 2010). Especially in *S. pombe*, PCR-based mutations or knock-out technologies are much more common than RNAi-based technologies and in that sense, direct introduction of siRNAs to *S. pombe* cells is a novel approach for gene expression research in yeast.

Non-viral siRNA transfer systems have been widely used in recent years. Amphiphilic molecules and nanoparticles (Ghosn *et al*., 2010) are the most prominent technologies among the transfer systems. Even though carrying siRNAs into the cells with nanoparticles shows increased efficiency, amphiphilic lipid molecules are being more widely performed, based on their ease of handling, low cost and adaptability (Conde *et al*., 2015).

In this study, three siRNAs were designed and synthesized complementary to 5'-UTR, exonic region and 3'-UTR of *spn1* gene. The siRNAs were introduced to cells via cationic liposomes and gene expression levels of *spn1* gene were analysed via Real-Time PCR. The highest decrease in *spn1* mRNA was observed in the cells that were introduced by the siRNA which is complementary to 3'-UTR of *spn1* gene. The expression of *spn1* gene was also reduced by the siRNA that is complementary to the exonic region. It has been shown that in fungi, specifically in Mucorcircinelloides, siRNAs can be derived from exons of genes (Nicolas *et al*., 2010) but this feature has not been documented in *S. pombe* before.

In order to investigate the interaction of siRNAs with each other, the combination of two siRNAs and all three siRNAs together were introduced to cells. The results revealed that all samples that have the siRNA with 3'-UTR complementarity reduced the level of expression more which also shows the potential of this specific siRNA. Cell growth curve results of siRNA applications supported

the findings from gene expression analysis and it is thought that the siRNA which was designed from the 3'-UTR of *spn1* gene could be a potential gene silencing agent via direct introduction of double-stranded siRNAs triggering the RNAi pathway in vitro in *S. pombe* cells. Depending on these data, cells were observed under fluorescent microscope for morphological changes. Long, chain-like cells with multiple nuclei were found following the siRNA application, thus proving the success of the silencing. These findings are in accordance with the previously documented observations with septin mutants which revealed that cell morphology changes based on arrested cytokines is that causes the formation of longer, chain-like and polynucleated cells (Wu *et al*., 2010).

Conclusion

This study plays an important role as a preliminary work for direct siRNA introduction to *S. pombe* cells which has not been documented before. For further analysis, specific antibodies for *Septin 1* can be applied in order to evaluate the protein expression of *Septin 1* gene after siRNA introduction and other types of *septins* should also be silenced via siRNAs in order to understand their combined effects in cells. The findings of this study are considered to lead a more intense investigation on siRNA research in *S. pombe* and the prospective outcomes of similar research will help to develop more structured use of synthetic siRNAs for gene silencing in yeast.

Acknowledgement

The authors thank Prof. Dr. Nazli Arda and Prof. Dr. Aysegul Topal Sarikaya for their scientific contributions to the project.

Funding Information

This research was funded by the Scientific Research Project Coordination Unit of Istanbul University, Project No: BAP 36503.

Author's Contributions

Semih Ekimler: Participated in all experiments, review literature and write manuscript.

Merve Yilmazer: Participated in all experiments.

Ercan Arican: Designed the research plan, organized the study and contributed to result analysis.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

An, H., J.L. Morrell, J.L. Jennings, A.J. Link and K.L. Gould, 2004. Requirements of fission yeast *septins* for complex formation, localization and function. Mol. Biol. Cell, 15: 5551-5564. PMID: 15385632

Bertin, A., M.A. McMurray, L. Thai, G. Garcia and V. Votin *et al*., 2010. Phosphatidylinositol-4,5-bisphosphate promotes budding yeast septin filament assembly and organization. J. Mol. Biol., 404: 711-731. PMID: 20951708

Bolotin-Fukuhara, M., B. Dumas and C. Gaillardin, 2010. Yeast as a model for human disease. FEMS Yeast Res., 10: 959-960. PMID: 18428391

Carthew, R.W. and E.J. Sontheimer, 2009. Origins and mechanisms of miRNAs and siRNAs. Cell, 136: 642-655. PMID: 19239886

Chen, S., X. Ge, Y. Chen, N. Lv and Z. Liu *et al*., 2013. Advances with Rna interference in Alzheimer's disease research. Drug Des. Devel. Ther., 7: 117-25. PMID: 23459401

Conde, J., E.R. Edelman and N. Artzi, 2015. Target-responsive DNA/Rna nanomaterials for microrna sensing and inhibition: The jack-of-all-trades in cancer nanotheranostics? Adv. Drug Deliv. Rev., 81: 169-183. DOI: 10.1016/j.addr.2014.09.003

Dang, Y., Q. Yang, Z. Xue and Y. Liu, 2011. Rna interference in fungi: Pathways, functions and applications. Eukaryot. Cell, 10: 1148-55. PMID: 21724934

Fields, S. and M. Johnston, 2005. Cell biology. Whither model organism research? Science, 307: 1885-1886. PMID: 15790833

Filipowicz, W., L. Jaskiewicz, F.A. Kolb and R.S. Pillai, 2005. Post-transcriptional gene silencing by siRNAs and miRNAs. Curr. Opin. Struct Biol., 15: 331-341. PMID: 15925505

Fire, A., S. Xu, M.K. Montgomery, S.A. Kostas and S.E. Driver *et al*., 1998. Potent and specific genetic interference by double-stranded RNA in caenorhabditis Elegans. Nature, 391: 806-811. PMID: 15925505

Forsburg, S.L. and N. Rhind, 2006. Basic methods for fission yeast. Yeast, 23: 173-183. PMID: 16498704

Ghildiyal, M. and P.D. Zamore, 2009. Small silencing rnas: An expanding universe. Nat. Rev. Genet, 10: 94-108. PMID: 19148191

Ghosn, B., A. Singh, M. Li, A.V. Vlassov and C. Burnett *et al*., 2010. Efficient gene silencing in lungs and liver using imidazolemodified chitosan as a nanocarrier for small interfering RNA. Oligonucleotides, 20: 163-172. PMID: 20565242

Gilden, J.K., S. Peck, Y.C. Chen and M.F. Krummel, 2012. The septin cytoskeleton facilitates membrane retraction during motility and blebbing. J. Cell Biol., 196: 103-114. PMID: 22232702

Goffeau, A., B.G. Barrell, H. Bussey, R.W. Davis and B. Dujon *et al*., 1996. Life with 6000 genes. Science, 274: 546-546. PMID: 8849441

Gutz, H., H. Heslot, U. Leupold and N. Loprieno, 1974, *Schizosaccharomyces pombe*. In: Handbook of Genetics, King, R.C. (Ed.), Plenum Press, New York, pp: 395-446.

Haarer, B.K. and J.R. Pringle, 1987. Immunofluorescence localization of the saccharomyces cerevisiae Cdc12 gene product to the vicinity of the 10-Nm filaments in the mother-bud neck. Mol. Cell Biol., 7: 3678-3687. PMID: 3316985

Hartwell, L.H., 1971. Genetic control of the cell division cycle in yeast: IV. Genes controlling bud emergence and cytokinesis. Exp. Cell Res., 69: 265-276. DOI: 10.1016/0014-4827(71)90223-0

Hu, J., X. Bai, J.R. Bowen, L. Dolat and F. Korobova *et al*., 2012. Septin-driven coordination of actin and microtubule remodeling regulates the collateral branching of axons. Curr. Biol., 22: 1109-1115. PMID: 22608511

Kanasty, R., J.R. Dorkin, A. Vegas and D. Anderson, 2013. Delivery materials for siRNA therapeutics. Nat. Mater, 12: 967-977. DOI: 10.1038/nmat3765

Kinoshita, M., 2003. The *septins*. Genome Biol., 4: 236-236.

Kinoshita, M., C.M. Field, M.L. Coughlin, A.F. Straight and T.J. Mitchison, 2002. Self- and actin-templated assembly of mammalian *septins*. Dev. Cell, 3: 791-802. PMID: 12479805

Li, L., S.S. Chang and Y. Liu, 2010. Rna interference pathways in filamentous fungi. Cell Mol. Life Sci., 67: 3849-3863. PMID: 20680389

Moreno, S., A. Klar and P. Nurse, 1991. Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. Methods Enzymol., 194: 795-823. DOI: 10.1016/0076-6879(91)94059-L

Mostowy, S. and P. Cossart, 2012. *Septins*: The fourth component of the cytoskeleton. Nat. Rev. Mol. Cell Biol., 13: 183-194. DOI: 10.1038/nrm3284

Mostowy, S., S. Janel, C. Forestier, C. Roduit and S. Kasas *et al*., 2011. A role for *septins* in the interaction between the listeria monocytogenes invasion protein InlB and the met receptor. Bioph. J., 100: 1949-1959. PMID: 21504731

Nicolas, F.E., S. Moxon, J.P. de Haro, S. Calo and I.V. Grigoriev *et al*., 2010. Endogenous short RNAs generated by Dicer 2 and RNA-dependent RNA polymerase 1 regulate mRNAs in the basal fungus Mucor circinelloides. Nucleic Acids Res., 38: 5535-5541. PMID: 20427422

Peterson, E.A. and E.M. Petty, 2010. Conquering the complex world of human *septins*: Implications for health and disease. Clin. Genet., 77: 511-524. PMID: 20236126

Pham, T., J.E. DiCiccio and W.S. Trimble, 2010. Cytoskeleton: *Septins* do the horizontal tango. Curr. Biol., 24: R324-R327. DOI: 10.1016/j.cub.2014.02.047

Reyes-Turcu, F.E. and S.I. Grewal, 2012. Different means, same end-heterochromatin formation by RNAi and RNAi-independent RNA processing factors in fission yeast. Curr. Opin. Genet. Dev., 22: 156-163. PMID: 22243696

Russell, S.E. and P.A. Hall, 2011. Septin genomics: A road less travelled. Biol. Chem., 392: 763-767. PMID: 21809895

Sellin, M.E., L. Sandblad, S. Stenmark and M. Gullberg, 2011. Deciphering the rules governing assembly order of mammalian septin complexes. Mol. Biol. Cell, 22: 3152-3164. PMID: 21737677

Sirajuddin, M., M. Farkasovsky, F. Hauer, D. Kuhlmann and I.G. Macara *et al*., 2007. Structural insight into filament formation by mammalian *septins*. Nature, 449: 311-315. PMID: 17637674

Spiliotis, E.T., S.J. Hunt, Q. Hu, M. Kinoshita and W.J. Nelson, 2008. Epithelial polarity requires septin coupling of vesicle transport to polyglutamylated microtubules. J. Cell Biol., 180: 295-303. PMID: 18209106

Vega, I.E. and S.C. Hsu, 2003. The septin protein Nedd5 associates with both the exocyst complex and microtubules and disruption of its gtpase activity promotes aberrant neurite sprouting in Pc12 cells. Neuroreport, 14: 31-37. PMID: 12544826

Weems, A.D., C.R. Johnson, J.L. Argueso and M.A. McMurray, 2014. Higher-order septin assembly is driven by GTP-promoted conformational changes: Evidence from unbiased mutational analysis in saccharomyces cerevisiae. Genetics, 196: 711-727. DOI: 10.1534/genetics.114.161182

Weirich, C.S., J.P. Erzberger and Y. Barral, 2008. The septin family of gtpases: Architecture and dynamics. Nat. Rev. Mol. Cell Biol., 9: 478-489. DOI: 10.1038/nrm2407

Wu, J.Q., Y. Ye, N. Wang, T.D. Pollard and J.R. Pringle, 2010. Cooperation between the *septins* and the actomyosin ring and role of a cell-integrity pathway during cell division in fission yeast. Genetics, 186: 897-915. PMID: 20739711