

The Role Of Some Chromatin Components In Chromosome Dynamics In *Arabidopsis thaliana* and Humans



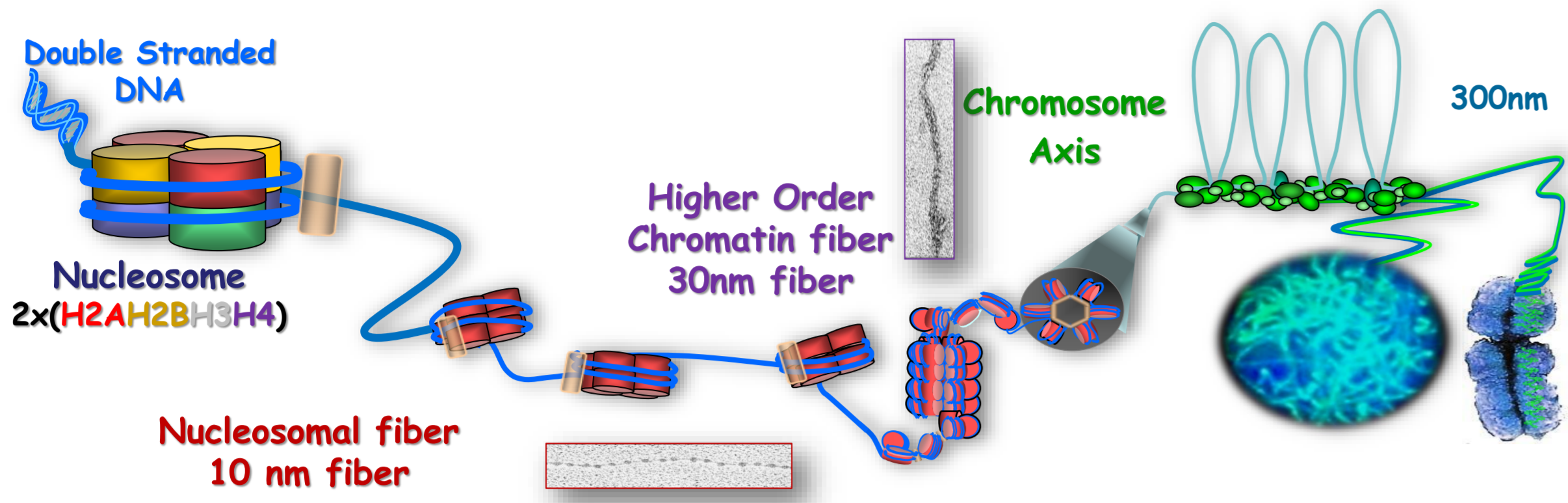
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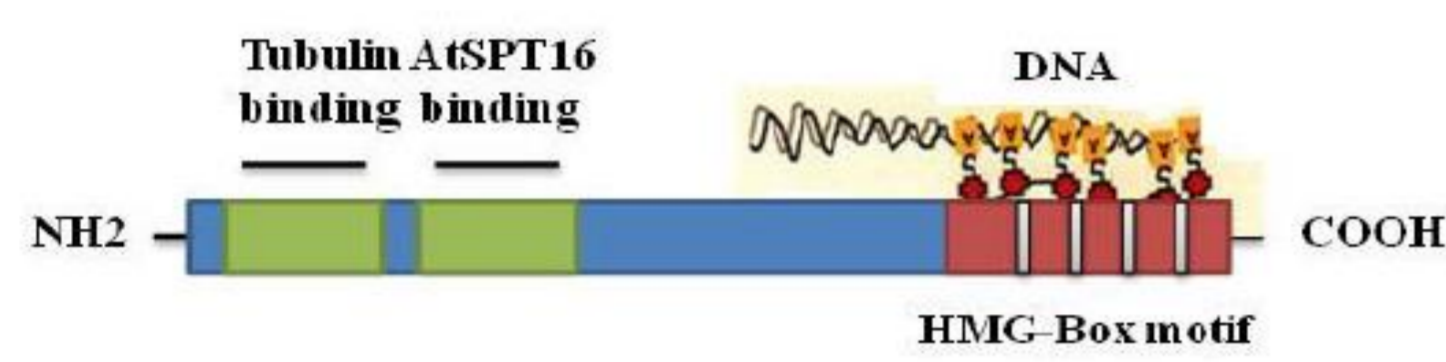
INTRUDUCTION



CHROMATIN STRUCTURE

- DNA associates with proteins to form the chromatin
- The classical model of chromatin involves a hierarchical compaction process¹
- The chromatin structure is highly dynamic and thus allows the vital activities of the nucleus²
- Chromatin compaction has been compared to "a riddle, wrapped in a mystery, inside an enigma³" because of the different controversial observations while studying its components
- The role of DNA is to store the genetic information of an individual
- DNA has to be packed in the cell nucleus in such a way that allows it to be accessible to carry out vital cellular processes¹

Structure-specific recognition protein 1 (SSRP1)



- Structure-specific recognition protein 1 (SSRP1) is a component of non-histone protein, High Mobility Group (HMG) protein family⁴
- The SSRP1 N-terminal domain interacts with protein SPT16 to form the FACT complex - Facilitates Chromatin Transcription
- SSRP1 seems to contain also a tubulin binding domain⁴
- SSRP1 C-terminal domain contains a DNA-binding motif
- SSRP1 has been reported to play a crucial role in DNA repair response
- SSRP1 might also be playing a role in Microtubules regulation^{4,5}

OBJECTIVES

- Analyse the complexity of chromatin in different organisms (Similarity, differences and the phenotype that can be observed as a result of mutating the same gene of different organisms)
- To investigate the role of different chromatin components and their interactions
- Analysis of chromatin components in different species (*Arabidopsis* and Humans)
- To investigate the role of SSRP1 in different species
- To characterize the chromatin defects (mutant lines) using different cytological and molecular analysis techniques

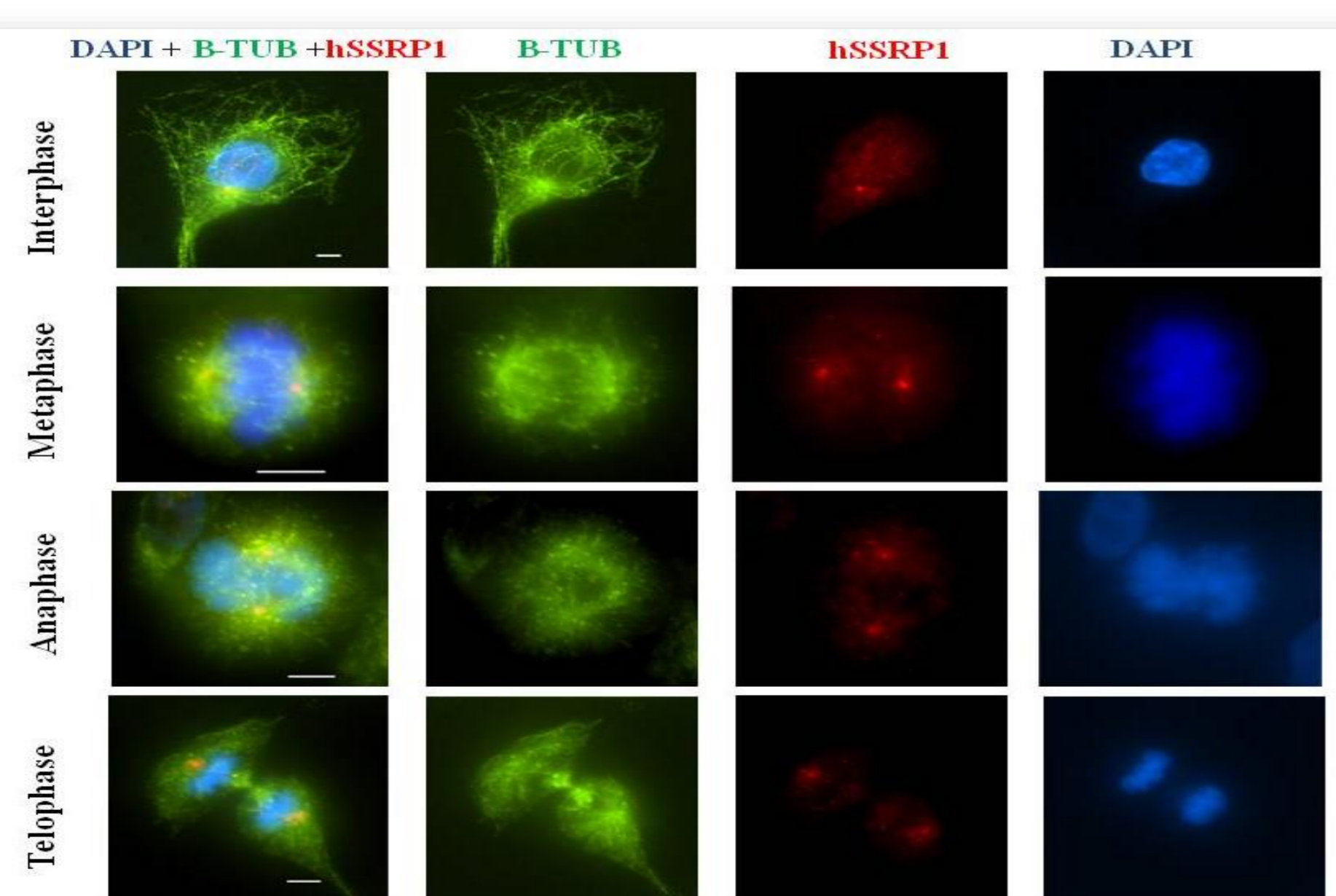
METHODS

- Spreading of *Arabidopsis* chromosomes
- Arabidopsis* Immunolocalization
- FLUTAX1 staining
- Human Cell Isolation and Tissue Culture
- siRNA Transfection of HUVEC
- Immunolocalization for human cells
- Small interference RNA (siRNA) knockdown mutation for human cell culture (Human Umbilical Vein Endothelial Cells (HUVEC))

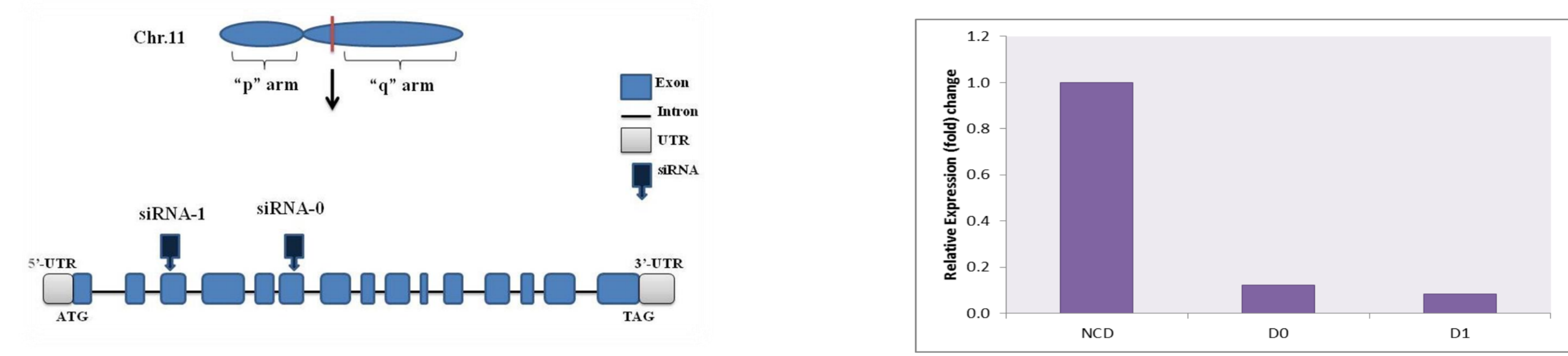
RESULTS

hSSRP1 LOCALISE AT THE CENTRIOLES

- Two different antibodies raised against human SSRP1 were used
- Both antibodies (raised in mouse and rabbit) provided identical localization of SSRP1 at the centrosome labelling two structures at each pole (similarly to centrioles)

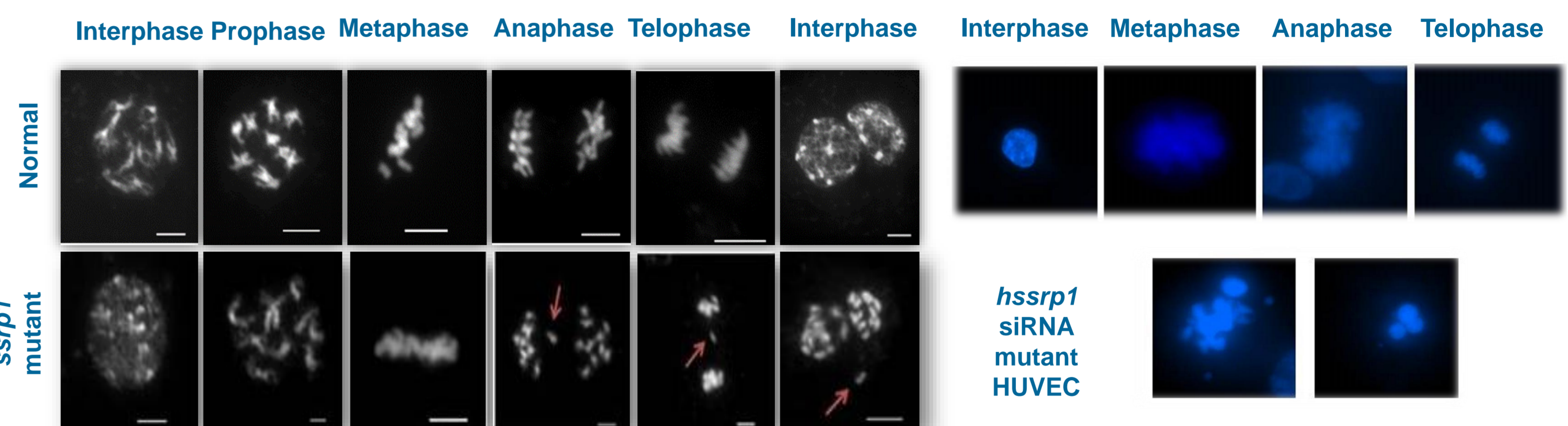


THE EFFICIENCY OF THE siRNA OLIGOS TO KNOCKDOWN hSSRP1 IN (HUVEC) WAS CONFIRMED USING REVERSE TRANSCRIPTASE QUANTITATIVE PCR (qPCR)

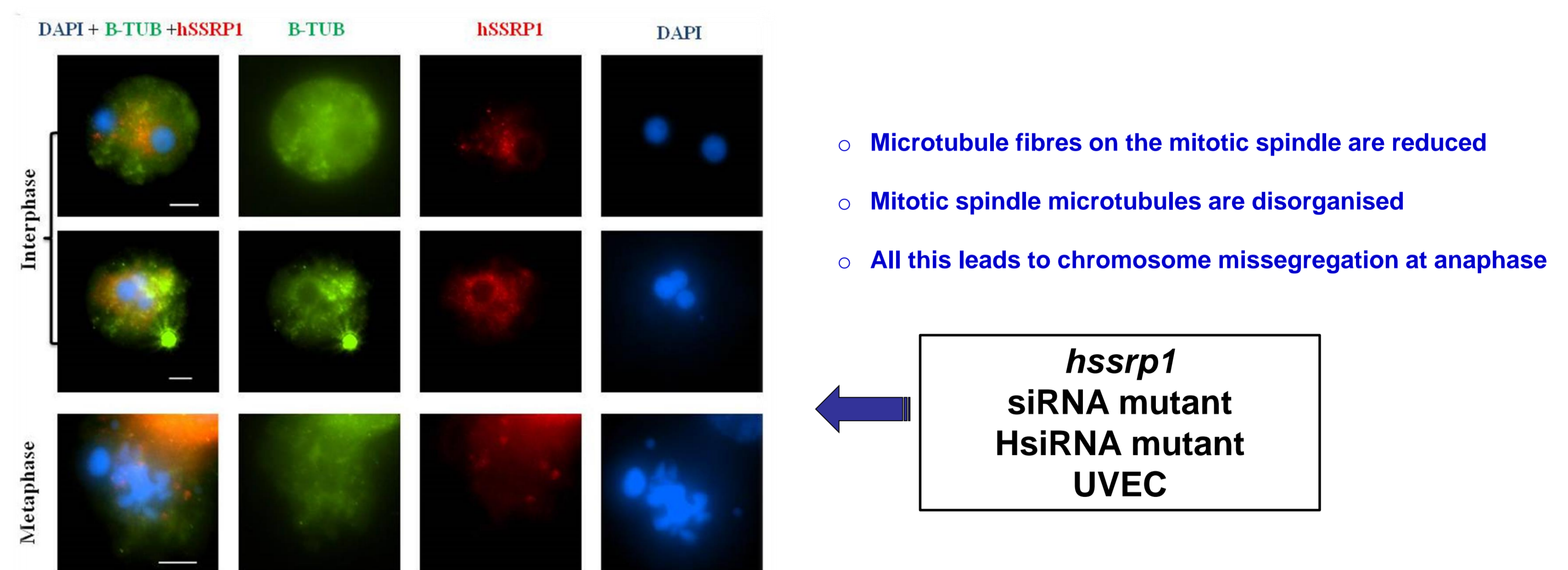


Expression of hSSRP1 by qPCR in HUVEC. Relative expression compared to beta-actin (*ACTB* gene). NCD; control duplex. D0; siRNA0. D1; siRNA1
Q-PCR analysis of *hssrp1* knockdown mutant cells

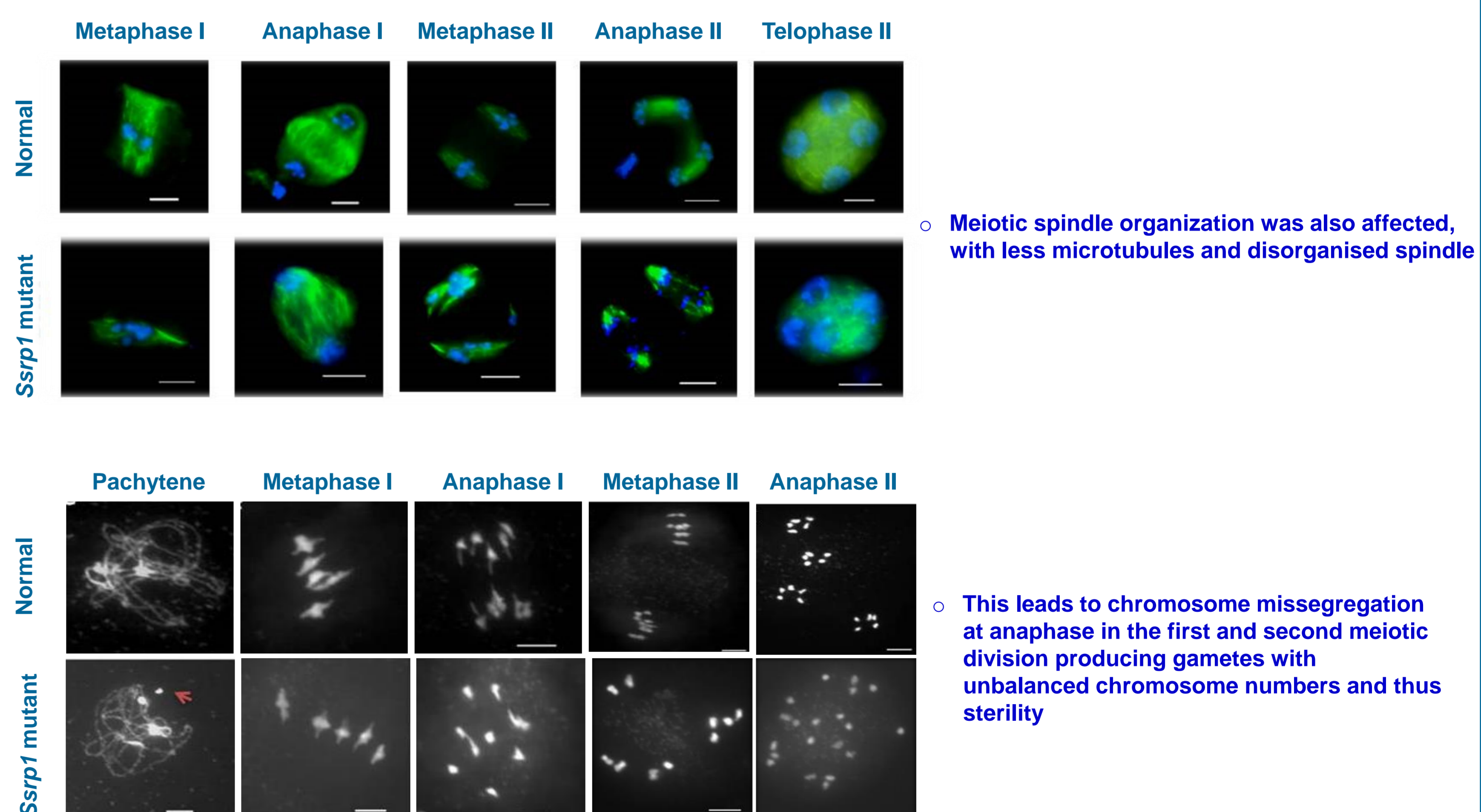
MITOTIC CHROMOSOME SEGREGATION IS PERTURBED IN SSRP1 MUTANT IN ARABIDOPSIS THALIANA AND HUMANS



IN THE ABSENCE OF SSRP1, MICROTUBULE SPINDLES ARE ABERRANT IN HUMANS



MEIOTIC MICROTUBULE SPINDLE ORGANIZATION IS ALSO ABERRANT IN ARABIDOPSIS THALIANA



CONCLUSIONS

- SSRP1 protein function is evolutionary conserved among plants and mammals
- SSRP1 mutants have showed chromosome instability during cell cycle and meiosis
- SSRP1 protein localises at the centrosome region in human mitotic spindle forming two structures similar to the centrioles
- In the absence of SSRP1 tubulin microtubules will lead to decrease in number at the mitotic and meiotic spindles which are not properly organised
- SSRP1 seems to have an important role in organizing the microtubules at the mitotic and meiotic spindles. And this function seems to be evolutionary conserved in humans and other species

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