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Localization and role of RAP55/LSM14 in HeLa cells: a new finding on the mitotic spindle assembly

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The MAP family includes large proteins like MAP-1A, MAP-1B, MAP-1C, MAP-2, and MAP-4 and smaller components like tau and MAP-2C. This article focuses on the relevant aspects of RAP55/LSM14 position with emphasis on its role in mitotic spindle formation and stability. In this context, the localization of RNA associated Protein 55kDa (RAP55/LSM14) during mitosis was identified as a Mitotic Spindle Protein (MSP). We found a new location obtained by expressing GFP-tagged proteins in HeLa Cells during mitosis that has never been previously reported. We demonstrated also, for the first time, that the depletion of RAP55/LSM14 destabilizes spindle assembly, stops cells in mitosis and induces many other cell cytoskeletal disorders. Finally, by using an "*in vitro*" assay investigation, we found that RAP55/LSM14 binds directly the tubulin and that is implicated in the process of the mitotic spindle stabilization, which is a novel discovery in this field of research.

Key words: LSM14, mitosis, microtubule, mRNP, RAP55, mitotic spindle

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INTRODUCTION

Microtubules (MTs) are cylindrical macromolecular structures composed of tubulin. They are found in the cytoplasm of all eukaryotic cells and they are the main components of the cell cytoskeleton. The main functions of MTs are (a) providing the mechanical stability of the cell, (b) transporting cellular material *via* motor proteins, (c) moving the cell while forming the interior of cilia or flagella, and (d) separating chromosomes during mitosis (Regnard *et al.*, 2003; Craddocka *et al.*, 2009).

MTs play fundamental roles in multiple cellular processes and there is a growing list of factors and molecular motors that regulate both, the organization and the spatiotemporal remodeling of the MT network.

Tubulin, the protein that constitutes MTs, is highly heterogeneous, especially in the C-terminal tail, which is important for the binding of most microtubule-associated proteins (MAPs) (Maccioni & Cambiazo, 1995).

RAP55 (LSM14a in humans) was identified among other MAPs and RNA-binding proteins. This protein attracted our attention because RAP55 was previously demonstrated to co-precipitate with Rae1, whose depletion prevented spindle formation in Xenopus egg extracts (Blower *et al.*, 2005; Marnefa *et al.*, 2009). Therefore, we used many techniques to further explore this protein localization during cell mitosis and to verify that this protein is a real component of the spindle-assembly machinery.

RAP55/LSM14 was first discovered in the newt *Pleurodeles waltl* and in *Xenopus laevis* as an RNA-associated protein of 55 kDa and initially thought to be oocyte-specific in its expression. A defining feature of RAP55/LSM14 is that it is a highly conserved 'LSM14 domain' present in a range of proteins called the LSM14 or Scd6 family. Eukaryotic RAP55/LSM14 proteins include Sum2p in *Schizosaccharomyces pombe*; Scd6 in *Saccharomyces cerevisiae*; CAR-1 in *Caenorhabditis elegans*; Trailer Hitch (Tral) in *Drosophila melanogaster*; xRAP55 in *X. laevis*; mTral in mouse and hRAP55/LSM14 in humans. In vertebrates, the family has further evolved into two RAP55/LSM14 protein paralogues, named RAP55A and RAP55B. To date, most studies have focused on RAP55A. However, former studies have demonstrated that RAP55/LSM14 is a constituent of the cytoplasmic processing bodies (P-bodies) formation, which are cytoplasmic structures detected in discrete foci and considered as sites of either mRNA decay or storage of non-translating mRNAs. Other recent studies have demonstrated that some constituents of the P-bodies are shuttled into stress granules (SGs), when mammalian cells are exposed to stress conditions (eg: heat shock) (Tanaka *et al.*, 2006). Some study, reported RAP55:LSM14 to be one of the autoantigens identified by the serum of patients with autoimmune diseases, primarily biliary cirrhosis. The functional properties and expression of RAP55A and RAP55B remain to be investigated in detail (Castoldi & Popov, 2003).

Therefore, to achieve our research goals, we have explored the RAP55/LSM14 localization in the HeLa cells using GFP-tagged proteins. Depletion of RAP55/LSM14 by siRNA reduces and destabilizes markedly the mitosis spindle.

We also investigated the effect of different concentrations of RAP55 in tubulin polymerization by a pull-down experiment (Sawin *et al.*, 1992).

MATERIALS AND METHODS

RAP55 Localization. To probe the role of RAP55/LSM14 in the spindle/microtubule assembly, we have to look first at the cellular localization of endogenous RAP55/LSM14. cDNAs corresponding to the identified protein were amplified by PCR using a high-fidelity thermostable DNA polymerase PhusionTM (Finnzymes)

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Abbreviations: MAPs, most microtubule-associated proteins; MTs, microtubules

for ten cycles in accordance with the manufacturer's instructions. Primers used for PCR are: Forward primer: 5'-TATGTCGACATGAGCGGGGGCA-3', Reverse primer: 5'-GAGGATCCTGCAGCAACTTTGTTG-3'. Amplified fragments were digested with appropriate restriction enzymes, gel-purified and ligated into a eukaryotic expression vector pHAT2. Recombinant proteins were produced in *E. coli* BL21 strain and purified using TalonTM resin (Clontech) according to the manufacturer's instructions.

Cell line. HeLa human carcinoma epithelial cell lines were obtained from the American type culture collection (ATCC. CCL-2). Cells were grown in DMEM supplemented with 10% FCS and antibiotics at 37°C. Plasmids were transfected into cells using LipofectamineTM (Invitrogen). Cells were observed 24–48 hours after transfection in Zeiss Axiovert 200M inverted microscope using a standard Alexa488/FITC bandpass filter. Images were acquired using CoolSnap HQ (Photometrics) black and white camera driven by the Metamorph software (Universal Imaging). DNA was stained with Hoechst 33258 at 10 μg/ml in PBST.

Role of RAP55/LSM14 in the mitotic spindle assembly. RAP55/LSM14 depletion. To probe this possibility, we depleted RAP55/LSM14 in HeLa cells with siRNA transfection.

siRNA transfection/Gene knockdown. Cell culture. HeLa cells were maintained at 37°C in Dulbecco's modified Eagle's medium supplemented with 10% calf serum. Cells grown on glass coverslips in a 24-well plate were transfected with small interfering RNA (siRNA) using Oligofectamine. The sense strands of siRNA used were RAP55 siRNA, 5-GUG-GGA-GUG-ACA-UUA-AAG-ACC-UU-3 and control siRNA (sequence not identified by the provider). 24, 48 and 72 hours after siRNA transfection, cells were fixed for immunocytochemistry. We explored the spindle aberrations by immunofluorescence microscopy and we verified the protein depletion by immunoblotting.

Immunofluorescence analysis. For immunofluorescence analysis, firstly, we used the cells that were already cultured and transfected on a glass coverslip in a 24-well plate. The cells were washed three times with phosphate-buffered saline (PBS) and then fixed with PBS containing 4% paraformaldehyde at room temperature for 30 min. The fixed cells were washed with PBS containing 0.1% Triton X-100 and then blocked with 1 mg/ml $NaBH_4$ in PBS. After having been blocked, the cells were incubated for 1 h with the primary antibodies, mouse monoclonal anti-α-tubulin (F2168 clone DM1A purified immunoglobulin, monoclonal anti-α-tubulin-FITC antibody produced in mouse, Sigma Aldrich) and rabbit polyclonal anti RAP55 (Santa Cruz Biotechnology, sc-398552). The cells were then washed three times with PBS containing 0.1% Triton X-100 and incubated with a secondary antibody, Alexa-fluor 488 anti-Mouse antibody (62197 Sigma Aldrich) and Alexa-fluor 568 anti-rabbit Antibody (SAB1102713 Sigma Aldrich) for 20 min, at room temperature and away from light. For DNA staining, cells were incubated with 5 μg/ml Hoechst 33342 for 5 min prior to fixation (861405 Sigma Aldrich). Glass coverslip was mounted on a glass slide with the Fluor save (Calbiochem 345789) and examined under a fluorescence microscope.

Western blotting (WB). The expression of RAP55 and tubulin was tested on WB in 50 μl of cell lysates using two antibodies: an anti-RAP55 and anti-α-tubulin from Sigma Aldrich.

Proteins were transferred onto a nitrocellulose membrane (Hybond-P, Amersham Biosciences), probed with a primary antibody and re-probed with the appropriate antibodies. The antigen–antibody complex was detected by incubation of the membranes for 1 h at room temperature with a peroxidase-coupled anti-IgG antibody and revealed using the ECL (Amersham Pharmacia Biotech) system. Blots were then exposed to film. The results obtained were observed in term of quantity.

RAP55/LSM14 and tubulin sedimentation. Tubulin purification. Tubulin purification from bovine brain was carried out as previously described by Castoldi & Popov, 2003 and Simpson *et al.*, 2000. Brains were cleaned (meninges elimination) and browed in a MES Buffer (MES 50 mM). Two cycles of polymerization and depolymerization were made in the presence of a high molarity PIPES buffer to eliminate the contaminant proteins like MAPs and molecular motors. The concentration of the final tubulin stock solution in BRB80 buffer (80 mM PIPES/KOH (pH 6.8), 1 mM $MgCl_2$, 1 mM EGTA), as determined by its absorbance at 280 nm (A_{280}) with $\varepsilon_{280} = 115\,000\ M^{-1}\ cm^{-1}$, was 25 mg/ml (250 μM). The OD measurements were done in the SpectraMax Plus Spectrophotometer (Molecular Devices).

RAP55/LSM14 purification. The pHAT2-xRAP55 plasmid, containing a T7-Lac promoter, was used to transform BL21. Star E-Coli (DE3) and then RAP55 was expressed and purified. This plasmid is in fact a pET-11 plasmid modification, it codes for a 6 Histidine tail which is useful for chromatography purification thanks to its affinity to Nickel.

The RAP55 pull-down assay. Tubulin/RAP55 interaction. In order to examine the interaction between RAP55/LSM14 and tubulin *in vitro*, 2 μg of RAP55 were incubated with 2, 4, 8, 16 μg of Microtubules in 50 μl of a reaction mixture containing BRB80 1X and GTP 1mM at room temperature for 30 min. The samples were analyzed by SDS-polyacrylamide gel electrophoresis (SDS/PAGE) and the gel was stained with Coomassie Brilliant blue R-250.

Recording of tubulin polymerization in presence of RAP55/LSM14. (a) Tubulin polymerization optimization: *in vitro*, tubulin assembly control was observed by measuring the change in light scattering at 350 nm (A350). The experiments were carried out in a Spectramax (Molecular Probes) spectrophotometer/plate reader with half-area 96-well plates (CLS3695; Corning). Tests with each RAP55/LSM14 concentration were performed in triplicate, with a total volume of 60 μl per well. As much as 250 μl of the assay mixtures, sufficient for the triplicates, were prepared on ice. The assay mixtures were prepared in BRB80 buffer and contained 40, 50 and 60 μM tubulin and 1mM GTP. We chose the 50 μM of tubulin as an optimal concentration. Then we have just added different concentrations of RAP55/LSM14 (1, 2, 4, 8 μM) to measure the tubulin polymerization in the presence of RAP55/LSM14). The plate was transferred to the reader, which was prewarmed to 37°C. The polymerization was measured every 30 s for 1 h, at 350 nm. Warming the plate to 37°C took about 2–3 min. To check the reversibility of the assembly, and to discriminate the turbidity from an unspecific protein precipitation, the plate was subsequently placed on ice, and turbidity was checked every 10 minutes for 30 minutes. Data analysis was performed with Microsoft Office Excel and SigmaPlot V8.02 equipped with Enzyme Kinetics Module V1.1.

(b) Tubulin polymerization in the presence of RAP55/LSM14 at different concentrations: 50 μM of

extracted tubulin was mixed with different concentrations of RAP55/LSM14 and the assay was performed in the presence of a single concentration of GTP. The values from individual samples were analyzed and plotted as a function of concentrations using Sigma Plot V8.02 equipped with Enzyme Kinetics Module V1.1.

Statistical analyses. One-way analysis of variance followed by Duncan test ($p < 0.05$) was performed. Data are expressed as a mean ± standard deviation (S.D.). Bivariate correlations between mitotic index and multinuclear cell frequency were analyzed by Pearson's test using XLSTAT software (XLSTAT, 2015, Addinsoft, New York, NY).

RESULTS AND DISCUSSION

Localization of RAP55/LSM14 as a novel spindle component

One of the objectives of this work was to identify a protein specific to mitotic spindles. To achieve this we selected one protein, which was uncharacterized or poorly studied and absent from MSP: RAP55. Thus, RAP55/LSM14 localization during mitosis was determined in a cervical cell line (HeLa) by expressing its cDNAs as a GFP-fusion and by indirect immune-fluorescence.

We used an anti-tubulin antibody that recognized polymerized α-tubulin. DNA was labelled with Hoechst 33258.

Figure1 shows spindles assembled in the presence of GFP-tagged candidate protein in live cells. GFP-RAP55/LSM14 was concentrated at the P-bodies, in the interphase. Indirect immunofluorescence showed that RAP55/LSM14 is clearly localized at the spindle during mitosis. Furthermore, during different mitosis stages: prophase, prometaphase, metaphase, anaphase and telophase, RAP55/LSM14 was observed at midbodies, spindles, asters, chromosomes and the P-bodies (Fig. 1).

Expression of GFP-tagged proteins makes it possible to observe protein localization in live cells although GFP-tagging may, in some cases, interfere with the correct localization of the expressed proteins. GFP-tagged versions of our novel candidate protein clearly localized to the mitotic spindles in live cells.

Some former studies have shown that LSM14/RAP55 is involved in mRNA storage in interphase cells (Tanaka *et al.*, 2006; Gache *et al.*, 2007). Although most mRNAs are silent in oocytes, some other studies have shown the evidence that specific mRNAs are localized at mitotic microtubules (Blower *et al.*, 2007) and some of these mRNAs are coding for key mitotic proteins, such as cyclin B1 (Groisman *et al.*, 2000).

The findings that spindles assembled in Xenopus egg extracts are extensively decorated by ribosomes (Liska *et al.*, 2004) support our theory. It is probable that LSM14/RAP55 play a role in the local regulation on the mitotic spindle.

Importance of RAP55/LSM14 in the mitotic spindle assembly

RAP55/LSM14 is required for spindle assembly: to define the role of RAP55/LSM14 in spindle assembly, we used siRNA-LSM14 to deplete RAP55/LSM14 and explore the effects on HeLa cells. Western blotting results confirmed LSM14 depletion (Fig. 2). We observed a remarkable drop in the RAP55/LSM14 quantity in the total protein extract between the HeLa cells and transfected HeLa cells. In fact, we were able to deplete 60% to 70% of RAP55/LSM14 compared with tubulin as a control (Fig. 2).

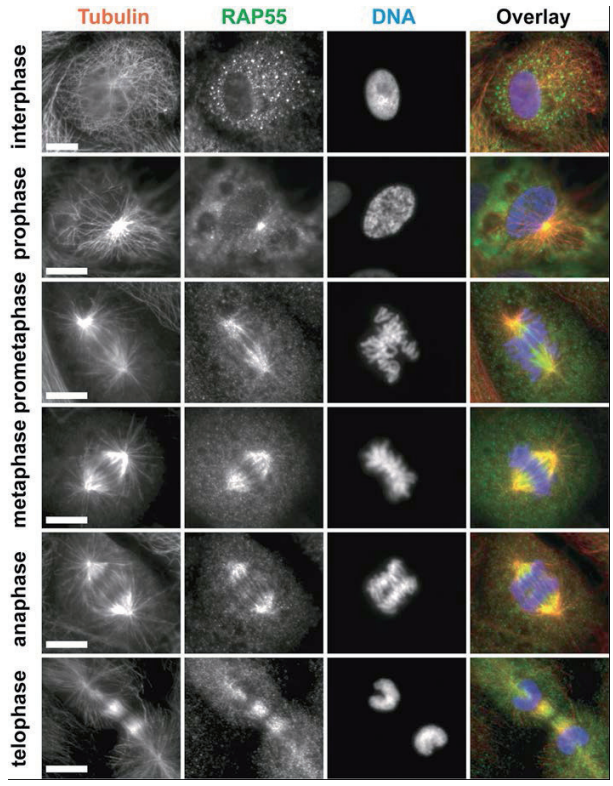

Figure 1. GFP-LSM14A during mitosis, α-tubulin tagged with IgG anti-tubulin, DNA stained with Hoechst 33258.

RAP55 depletion induced 40% of cells arrested in mitosis, as a result of a defect in the spindle assembly. A significant increase in multipolar spindles (20% of mitotic cells) was also observed (Fig. 3a). Epifluorescence microscopy showed many other spindle aberrations: chromosome failure to align properly in the prometaphase/metaphase, spindle with shifted chromosome, spindle with reduced density of MT and a mini-spindle. The extra spindle poles seemed to be functional, as they appeared to pull chromosomes away from the main spindle leading to the formation of multinuclear cells (Fig. 4).

The existence of giant cells can be explained by a supposed effect of LSM14 on actin function (Dominguez & Holmes, 2011) (Fig. 4).

The observed results suggest that RAP55/LSM14 plays an important role in microtubule stabilization in mitosis. Thus, RAP55 is required for proper spindle assembly, chromosome segregation and microtubule stabilization in human somatic cells.

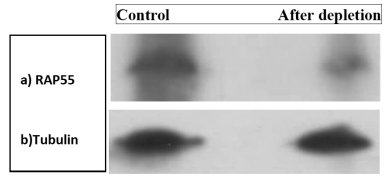

Figure 2. Western immunoblotting: (a) RAP55 in control HeLa cells and RAP55 after siRNA-LSM14 transfection in HeLa cells (b) the blot was probed for tubulin.

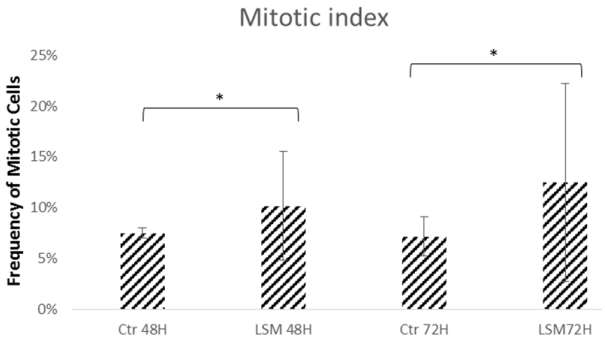

Figure 3a. Quantification of mitotic phenotypes observed after LSM14 knockdown.
Ctr, Control cells; LSM, siRNA-LSM14 transfected cells. The average value ±S.D. from three independent experiments is also shown. Asterisks indicate significant differences (*$p < 0.05$) calculated by the Duncan's *t*-test.

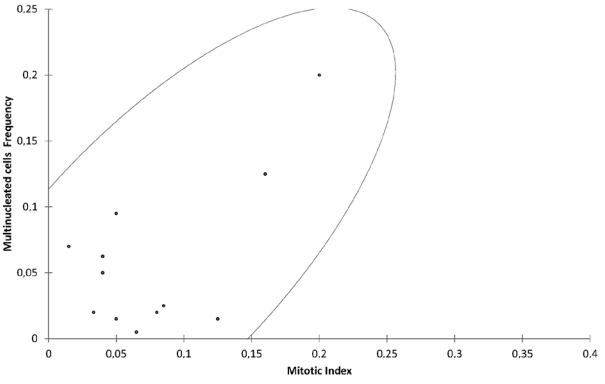

Figure 3c. Pearson's correlation = +0.5043, $p < 0.05$.
This correlation was more pronounced after 72 h of the assay (Pearson's correlation = +0.7342, $p < 0.05$) compared to the 48 h (Pearson's correlation = +0.321, $p < 0.05$).

Mitotic frequency and multinuclear HeLa cells after siRNA-LSM14/RAP55 transfection

Mitotic and multinuclear cells were counted in 10 viewing fields for each experimental condition, with a Zeiss Axiovert 200M inverted microscope (400×).

The expression of RAP55/LSM14 was inhibited by transfection with specific siRNAs, which targeted this protein, and its activity was subsequently evaluated in these siRNA transfected cells. Figure 3a shows the evaluation of the quantification of mitotic cells observed after the RAP55/LSM14 knockdown. After 48 h of siRNA-LSM14 transfection, 11% of HeLa cells were blocked in mitosis and 13% after 72 h. Using Duncan test ($p < 0.05$) these frequencies showed a significant increase compared to the control cells (5%). Therefore, the knockdown of RAP55/LSM14 induced the cell block in mitosis. The mitotic arrest effect of RAP55/LSM14 is also time dependent; the number of mitotic cells is increasing during time.

This experiment and the protein localization suggest that LSM14/RAP55 is directly implicated in the mitotic spindle polymerization and/or its organization during mitosis. Figure 3b, shows an increasing number of multinuclear cells following the RAP55/LSM14 knockdown.

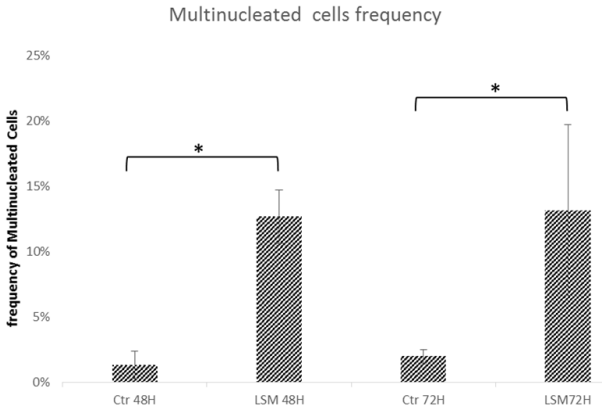

Figure 3b. Quantification of multinucleated cells observed after LSM14 knockdown.
Ctr, Control cells; LSM, siRNA-LSM14 transfected cells. The average value ±S.D. from three independent experiments is also shown. Asterisks indicate significant differences (*$p < 0.05$) calculated by the Duncan's t-test.

After 48H of siRNA-LSM14 transfection, 10% of HeLa cells became multinuclear and 12% after 72 h instead of 0.5% and 2% for the control cells. The absence of RAP55/LSM14 protein induced the transformation of HeLa cells into multinuclear ones. In fact, a positive correlation was observed between the mitotic index and the multinuclear cell frequency (Pearson's correlation = +0.5043, $p < 0.05$). This correlation was more appreciated after 72 h of the assay (Pearson's correlation = +0.7342, $p < 0.05$) compared with the 48 h (Pearson's correlation = +0.321, $p < 0.05$). This aberration (multinuclear cells) is known to result from abnormal mitosis. Therefore, in accordance to the localization of LSM14/RAP55 at the

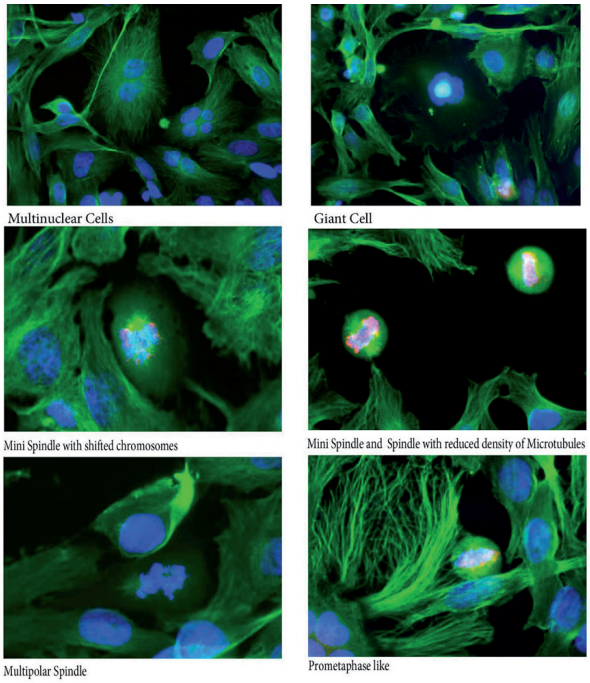

Figure 4. Spindle perturbation in HeLa cells after LSM14 depletion.
Tubulin was tagged with IgG anti-tubulin (green fluorescence), DNA was labelled with Hoechst dye. Scale bar 10 μm.

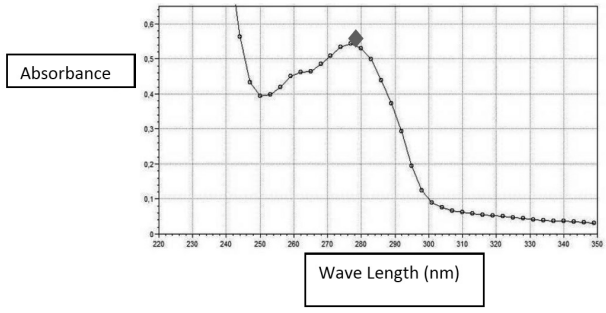

Figure 5. Optical density of a tubulin sample diluted 1:100; the red diamond indicates the 280 nm absorption.

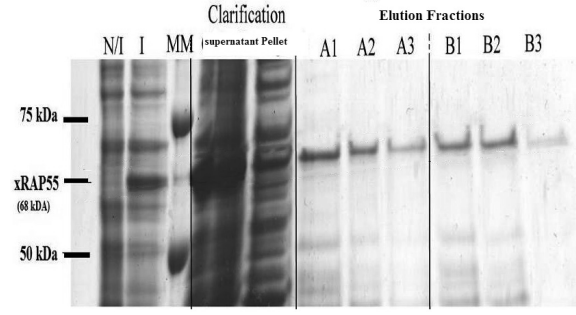

Figure 6. SDS/PAGE gel shows RAP55 purification.
NB, RAP55 migrates with a 68 kDa as a molecular weight. N/I, Non-induced;, MM, Molecular weight marker; A (1–3), Non-treated eluate; B (1–3), eluate treated with RNAse (1 mg/ml).

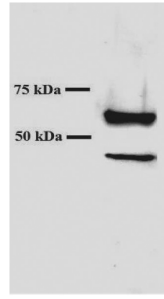

Figure 7. Two distinguished bands of RAP55 recognized by a specific anti- RAP55 antibody.

mitotic spindle and its effect on the blocking of mitosis, we can strongly suggest that this protein is implicated in the mitotic spindle formation and in chromosome segregation during mitosis.

Obviously, siRNA-LSM14 transfection clearly elevates the mitotic defect and multinuclear cell frequency.

A consequence of mitotic abnormalities induced by RAP55 depletion could be the instability of mitotic spindle. To verify this theory, we proceeded to tubulin-RAP55 sedimentation/pull-down and polymerization assay *in vitro*.

RAP55 and tubulin sedimentation: to determine if tubulin interacts directly with RAP55, co-precipitation study was performed as follows.

Tubulin purification: bovine brain tubulin was quantified and analyzed by SDS/PAGE electrophoresis. The final concentration was estimated to be 207 μM (Fig. 5). According to SDS-gel electrophoresis, the tubulin consisting of α, β-heterodimers was >99% pure and free of microtubule-associated proteins.

RAP55 purification: After extraction and purification of RAP55 from BL21 STAR *E. coli*, we ran our elution fraction on an SDS/PAGE gel (Fig. 6) and then we performed a western-blot to inspect the purity of our protein.

Then we used a polyclonal anti-RAP55 antibody that recognizes a peptide corresponding to RAP55 C-terminal domain (Tanaka *et al.*, 2006; Simpson *et al.*, 2006). The result showed that our purified protein is indeed RAP55 (Fig. 7).

Tubulin-RAP55 precipitation: fractions obtained in the performed tubulin-RAP55 pull-down assay were run on an SDS/PAGE gel and stained with Coomassie Blue. The results showed that in the lanes containing purified tubulin, a band that reacted with the RAP55 protein was seen at the expected position in the gel (Fig. 8, lane P1, P2, P3, and P4). The size of the band on SDS/PAGE in each lane was proportional to the quantity of tubulin linked to RAP55/LSM14. The LSM14/RAP55-tubulin binding was obvious. RAP55/LSM14 is in fact a tubulin binding protein required for regular spindle assembly.

RAP55 functions to Nucleate/Stabilize Microtubules

Tubulin/RAP55 interaction: as shown in Fig. 9, the optimal concentration of tubulin during polymerization is 50 μM; thus, we adopted it for the rest of the tests (Fig. 9).

Clearly, we observed that the addition of different concentrations of RAP55/LSM14 as a crude protein, did not markedly affect the tubulin polymerization; in fact, tubulin polymerization was almost stable during the course of the experiment (Fig. 10). This result leads to suggest that other proteins are necessary for RAP55/LSM14 to exert its full function on tubulin and in consequence microtubules. Those proteins could be other MAPs or motors. As a MT-stabilizing agent, RAP55/LSM14 promotes correct spindle assembly and inhibits aberrations during cell mitosis. The localization of RAP55/LSM14, as demonstrated earlier, was concentrated in the P-bodies around the nucleus during the interphase. In mitotic cells, RAP55/

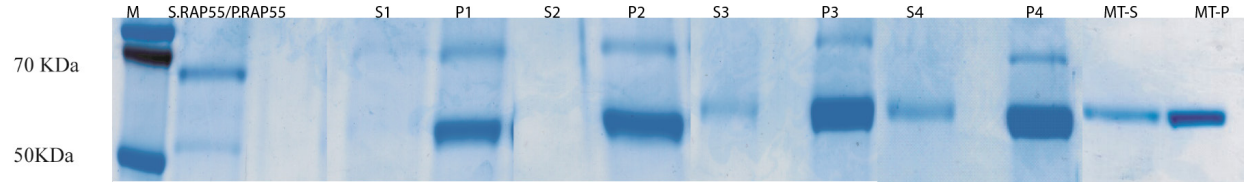

Figure 8. Pull-down experiments of tubulin and RAP55.
M, Molecular weight marker; S, supernatant; P, pellet; MT, microtubule. MT-S, Supernatant of MT; MT-P, Pellet of MT. S1, P1, supernatant and pellet obtained after the addition of 2 μg of MT. S2, P2, supernatant and pellet obtained after the addition of 4 μg MT. S3, P3, supernatant and pellet obtained after addition of 8 μg MT. S4, P4, supernatant and pellet obtained after the addition of 16 μg MT.

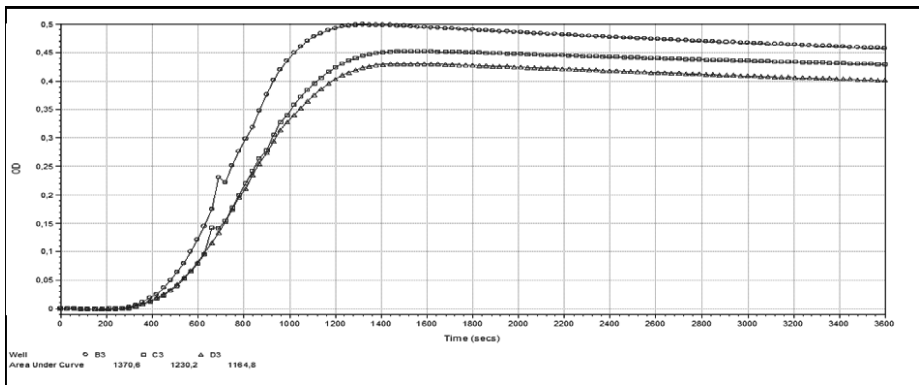

Figure 9. Kinetic measurements of tubulin samples performed with a spectrophotometer.

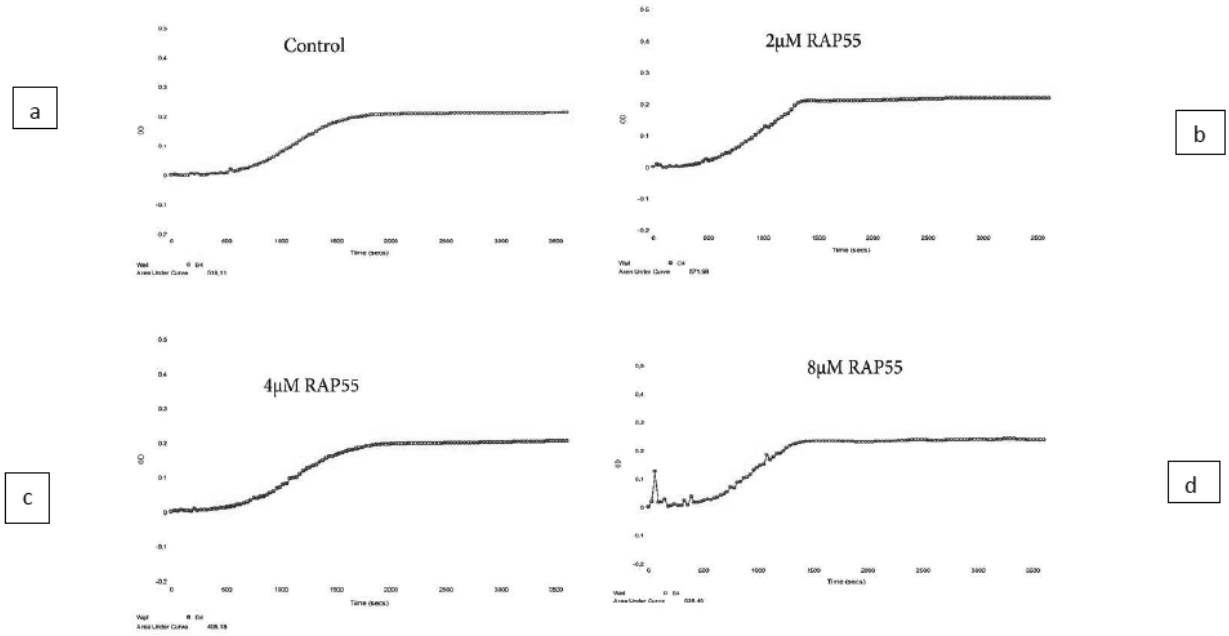

Figure 10. Tubulin polymerization essay in the presence of different RAP55 concentrations
(**a**) Control assay: 50 μM tubulin + 1 mM GTP, (**b**) 50 μM tubulin + 1mM GTP + 2 μM RAP55, (**c**) 50 μM tubulin + 1 mM GTP + 4 μM RAP55, (**d**) 50 μM tubulin + 1 mM GTP + 8 μM RAP55.

LSM14 is present at spindles, chromosomes and asters, and its requirement for the spindle assembly suggested that RAP55/LSM14 binds to microtubules. Former studies (Blower *et al.*, 2007; Cha *et al.*, 1999) confirmed that the overexpression of RAP55/LSM14 induced a pronounced microtubule bundling in interphase cells, frequently observed with proteins directly binding to microtubules. To rule out the possibility that chromosomal abnormalities induced by RAP55/LSM14 were due to direct binding of tubulins and thereby interfering with its polymerization or depolymerization, we conducted *in vitro* polymerization reactions using tubulin and RAP55/LSM14.

The results of this paper shows that RAP55/LSM14 by itself has only limited effects on the kinetics of tubulin polymerization (Fig. 10). We therefore suggest that RAP55/LSM14 requires additional factors for activity.

CONCLUSION

Our study has demonstrated that RAP55/LSM14 takes part in human cell mitosis. After examining the consequences of RAP55/LSM14 depletion in cells during mitosis, we noticed an accumulation of spindle abnormalities in the pre-anaphase state. These aberrations are characterized by the absence of focused poles, and chromosomes that do not become stably attached to the spindle. Some former studies have revealed similar results with NuMA, which is a nuclear protein. They have found large aberrant microtubules in the depleted extracts. In addition of its localization near the microtubules minus end, NuMA is considered as a nucleated protein for the microtubules (Merdes *et al.*, 1996). Based on this study and others, we suppose that RAP55/LSM14 have the same essential role in spindle assembly during mitosis. RAP55/LSM14 stabilizes spindle during mitosis and in harmony with its localization during mitosis and its direct binding with tubulin, we confirm that this protein is a nucleated protein, and is one of the most important MAPs studied so far.

Several recent proteomic studies have aimed to characterize proteomes related to the microtubule cytoskeleton (Bakhoum *et al.*, 2009; Gache *et al.*, 2010).

In fact, most of the known spindle proteins have an important role in its function. Some of the few, but notable exceptions include XMAP230, XMAP215, RAP55whose depletion from egg extracts does not significantly perturb spindle formation in this system. (Craddocka *et al.*, 2009; Tanaka *et al.*, 2006; Gache *et al.*, 2007; Cha *et al.*, 1999).

Thus, by virtue of their localization, any newly found spindle components automatically become candidate regulators of the spindle assembly and potential targets for anti-mitotic drugs (Magalhaes *et al.*, 2013).

This is why RAP55/LSM14 is a potential target for cancer therapy research (Craddocka *et al.*, 2009). This concept has been substantiated by the observations that suppression of RAP55/LSM14 expression or activity by anti-sense oligonucleotides (siRNAs) or dominant negative mutants induces programmed cell death in human tumors, which is often preceded by abnormal spindle formation (Blower *et al.*, 2007; Gumireddy *et al.*, 2005). Thus, it may serve as a promising antitumor target, particularly for patients with drug resistant tumors (Cai *et al.*, 2013).

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REFERENCES

Bakhoum SF, Thompson SL, Manning AL, Compton DA (2009) *Nature Cell Biol* **11**: 27–35.
Blower MD, Brown KS, Maresca TJ, Grammer TC, Harland RM, Heald R (2005) *J Cell Biol* **6**: 765.
Blower MD, Feric E, Weis K, Heald R (2007) *J Cell Biol* **179**: 1365–1373.
Cai P, Lu P, Sharom FJ, Fang WS (2013) *Cancer Lett* **1**: 214–223.
Castoldi M, Popov AV (2003) *Protein Expr Purif* **32**: 83–88.
Cha BJ, Cassimeris L, Gard DL (1999) *J Cell Sci* **112**: 4337–4346.
Craddocka TJA, Beaucheminb C, Tuszynskia JA (2009) *BioSystems* **97**: 28–34.
Dominguez R, Holmes KC (2011) *Annu Rev Biophys* **40**: 169–186.
Gache V, Waridel P, Luche S, Shevchenko A, Popov AV (2007) *Methods Mol Med* **137**: 29–43.
Gache V, Waridel P, Winter C, Juhem A, Schroeder M, Shevchenko A, Popov AV (2010) *PLoS One* **5**: e9248.
Groisman I, Huang YS, Mendez R, Cao Q, Theurkauf W, Richter JD (2000) *Cell* **103**: 435–447.
Gumireddy K, Ramana MVR, Cosenza SC, Nathan RB, Baker SJ, Papathi N, Jiang J, Holland J, Reddy EP (2005) *Cancer Cell* **7**: 497.
Liska AJ, Popov AV, Sunyaev S, Coughlin P, Habermann B, Shevchenko A, Bork P, Karsenti E, Shevchenko A (2004) *Proteomics* **4**: 2707–2721.
Maccioni RB, Cambiazo V (1995) *Physiological Rev* **75**: 835–864.
Magalhaes HI, Wilke DV, Bezerra DP, Cavalcanti BC, Rotta R, de Lima DP, Beatriz A, Moraes MO, Diniz-Filho J, Pessoa C (2013) *Toxicol Appl Pharmacol* **272**: 117–126.
Marnefa A, Sommervilleb J, Ladomeryc MR (2009) *Int J Biochem Cell Biol* **41**: 977–981.
Merdes A, Ramyar K, Vechio JD, Cleveland DW (1996) *Cell* **87**: 447–458.
Regnard C, Fesquet D, Janke C, Boucher D, Desbruyeres E, Koulakoff A, Insina C, Travo P, Edde B (2003) *J Cell Sci* **116**: 4181–4190.
Sawin KE, LeGuellec K, Philippe M, Mitchison TJ (1992) *Nature* **359**: 540–543.
Simpson JC, Nilsson T, Pepperkok R (2006) *Mol Biol Cell* **17**: 723–737.
Simpson JC, Wellenreuther R, Poustka A, Pepperkok R, Wiemann S (2000) *EMBO Rep* **1**: 287–292.
Tanaka KJ, Ogawa K, Takagi M, Imamoto N, Matsumoto K, Tsujimoto M (2006) *J Biol Chem* **281**: 40096–40106.