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Translational repression by SESA is growth medium dependent

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The correct separation of chromosomes during mitosis is necessary to prevent genetic instability and aneuploidy which causes cancer, and other diseases. The main criteria for this is the correct duplication of the centrosome. Recently, we reported that Smy2 can suppress the essential role of *MPS2* in the insertion of yeast centrosome into the nuclear membrane and co-operates with Eap1, Scp160, Asc1 for this task. We gave the name SESA (Smy2, Eap1, Scp160, Asc1) network to the system consisting of these four proteins. Detailed analysis showed that the SESA system is part of a mechanism which regulates translation of *POM34* mRNA. Thus, SESA, is a system which suppresses spindle pole body (SPB) duplication defects by inhibiting the translation of *POM34* mRNA (Sezen, 2009). Although many important points regarding SESA network have been discovered, many others remain obscure. How SESA system is activated, what is the trigger factor for Pom34 down regulation remains to be solved. In an attempt to uncover this, we came up with a surprising finding that translational repression of Pom34 by the SESA network is dependent on the growth medium. SESA is active when cells are grown in YPDA, but inactive in SC-Leu medium.

Keywords: ***Saccharomyces cerevisiae;*** **centrosome duplication; SESA network; growth media**

INTRODUCTION

In animal cells, the nuclear membrane disintegrates as they enter mitosis, revitalizes as they exit. In fungal cells such as *Saccharomyces cerevisiae*, that go through closed mitosis, the nuclear membrane remains intact throughout the entire cell cycle. Closed mitosis is possible because the yeast centrosome (SPB) is embedded in the nuclear membrane, allowing both cytoplasmic and nuclear microtubules to be secreted. The centrosomes are duplicated in the G1 phase of the cell cycle and settle into the nuclear membrane as a part of the duplication process. It has been shown that *NDC1*, *MPS2*, *BBP1* and *NBP1* gene products are required for the insertion of the centrosome into the nuclear membrane (Jaspersen and Winey, 2004).

The SPB duplication requires a mechanism for insertion of the new SPB into the nuclear membrane (Jaspersen and Winey, 2004). Mps2 is a single membrane spanning protein that inserts the newly formed centrosome into the nuclear envelope in yeast cells (Winey, 1991; Munoz-Centeno, 1999; Schramm, 2000; Araki, 2006; and Jaspersen, 2006). We previously identified *SMY2* as a high dosage suppressor for the lethal phenotype of the deletion of *MPS2* and reported that *mps2*Δ 2µm-*SMY2* cells are viable (Sezen, 2009). Furthermore, we demonstrated that the gene products of *SMY2*, *EAP1*, *SCP160* and *ASC1* closely cooperate in the suppression of *MPS2* deletion. The network of genes, named SESA, is responsible for the survival of *mps2*Δ 2µm-*SMY2* cells (Sezen, 2009). *POM34* mRNA associates with the SESA network of proteins.

Pom34 protein level is significantly reduced in *mps2*Δ 2µm-*SMY2* cells with respect to wild type cells. Thus, reduced translation of *POM34* by the SESA network suppresses SPB duplication failure caused by the loss of *MPS2*.

Although many components of the SESA network were discovered, we do not know the exact mechanism how SESA is activated, its trigger factor is not known yet. In this study, we wanted to analyze dependence of SESA activity on the Smy2 levels and test whether overexpression of Smy2 is sufficient for turning on the SESA activity or not. We also tested the dependence of SESA activity on variations in growth media, in order to have a hint for the SESA activation process.

MATERIALS AND METHODS

Preparation of Growth Media

SC-Leu Medium is prepared by dissolving 6.7 g Bacto-yeast nitrogen base w/o amino acids, 20 g glucose and 2 g SC-Leu drop out mix (mixture of same amounts of adenine, alanine, arginine, asparagines, aspartate, cysteine, glutamine, glutamate, glycine, histidine, inositol, isoleucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, uracil, valine, para-aminobenzoic acid) in 1 l final volume of dH_2O, and autoclaved.

YPDA Medium is prepared by dissolving 10 g Bacto-yeast extract, 20 g Bacto-peptone, 20 g glucose and 100 mg Adenine in 1 l final volume of dH_2O, and autoclaved.

Preparation of Yeast Cell Lysates using TCA for SDS-PAGE

To prepare whole cell lysates, 1-3 OD_{600}s were harvested from an overnight culture. The cell pellets were resuspended in 800 µl dH_2O, 150 µl 1.85 M sodium hydroxide and mixed by vortexing. The samples were then incubated on ice for 10 min and 150 µl TCA added, followed by vortexing and a further 10 min incubation on ice. The precipitated protein was pelleted by centrifugation at 14,000 rpm for 15 min at 4°C. The supernatant was removed, the samples spun again briefly and the residual supernatant discarded. The protein pellets were resuspended in 100-150 µl HU buffer (depending on pellet size and protein abundance), heated at 65°C for 15 min and centrifuged at 14,000 rpm for 10 min. Samples were then analysed by SDS-PAGE and Western blotting.

DAPI Staining

DNA positions of cells were visualized by DAPI staining. Cells from 1 ml culture were sedimented and re-suspended in 70% ethanol. The sample can be stored at 4°C for days. Cells were washed and re-suspended in 10-30 µl of 0.1 µg/ml DAPI in PBS.

FACS Analysis

The flow analysis cytometry system (FACS) was used to measure the DNA content of yeast cells. This is also a good indicator of the cell cycle stage.

5 ml of yeast culture (OD_{600} 0.4-1.0) was harvested and the cell sediment was resuspended in 2 ml 70% ethanol. This was mixed on a rotating wheel overnight. Cells were sedimented at 1,000 rpm for 5 min and resuspended in 0.5 ml 50 mM Tris-HCl pH 7.8 before transfer to a 2 ml tube. Cells were washed once with 1 ml 50 mM Tris-HCl pH 7.8 (centrifugation for 10 sec). Cells were re-suspended in 800 µl Tris-HCl pH 7.8 and 200 µl 10 mg/ml RNaseA added (boiled for 20 min and centrifuged at 14,000 rpm for 5 min). Samples were incubated on roller at 37°C overnight. Cells were sedimented, resuspended in 0.5 ml 0.5 mg/ml pepsin (in 55 mM HCl) and incubated in 37°C water bath for 45 min. Cells were washed with 1 ml FACS buffer and then resuspended in 0.5 ml FACS buffer. To this suspension 5 µl 1 mg/µl propidium iodide was added.

To read the samples on the FACS machine the samples were first sonicated for 10 sec in a water bath sonicator, 20,000 events were read per samples and files were processed using the Cell Quest program.

RESULTS

In this study, we wanted to gain some insight about the activation step for the action of SESA network and first focused on the phenotypic characterization of *mps2*Δ 2µm-*SMY2* mutant cells. *mps2*Δ 2µm-*SMY2* cells showed a general delay in cell cycle progression as bud formation, DNA replication and anaphase onset were delayed by 20-30 min compared to wild type cells (Figure 1). Surprisingly, at 23ºC SPB duplication was not strongly defective in *mps2*Δ 2µm-*SMY2* cells as judged by the separation of the Spc42-eqFP611 SPB marker (Sezen, 2009). Moreover, components of the Mps2-Bbp1-Nbp1-Ndc1 machinery showed normal localization to the SPBs of *mps2*Δ 2µm-*SMY2* cells (Sezen, 2009).

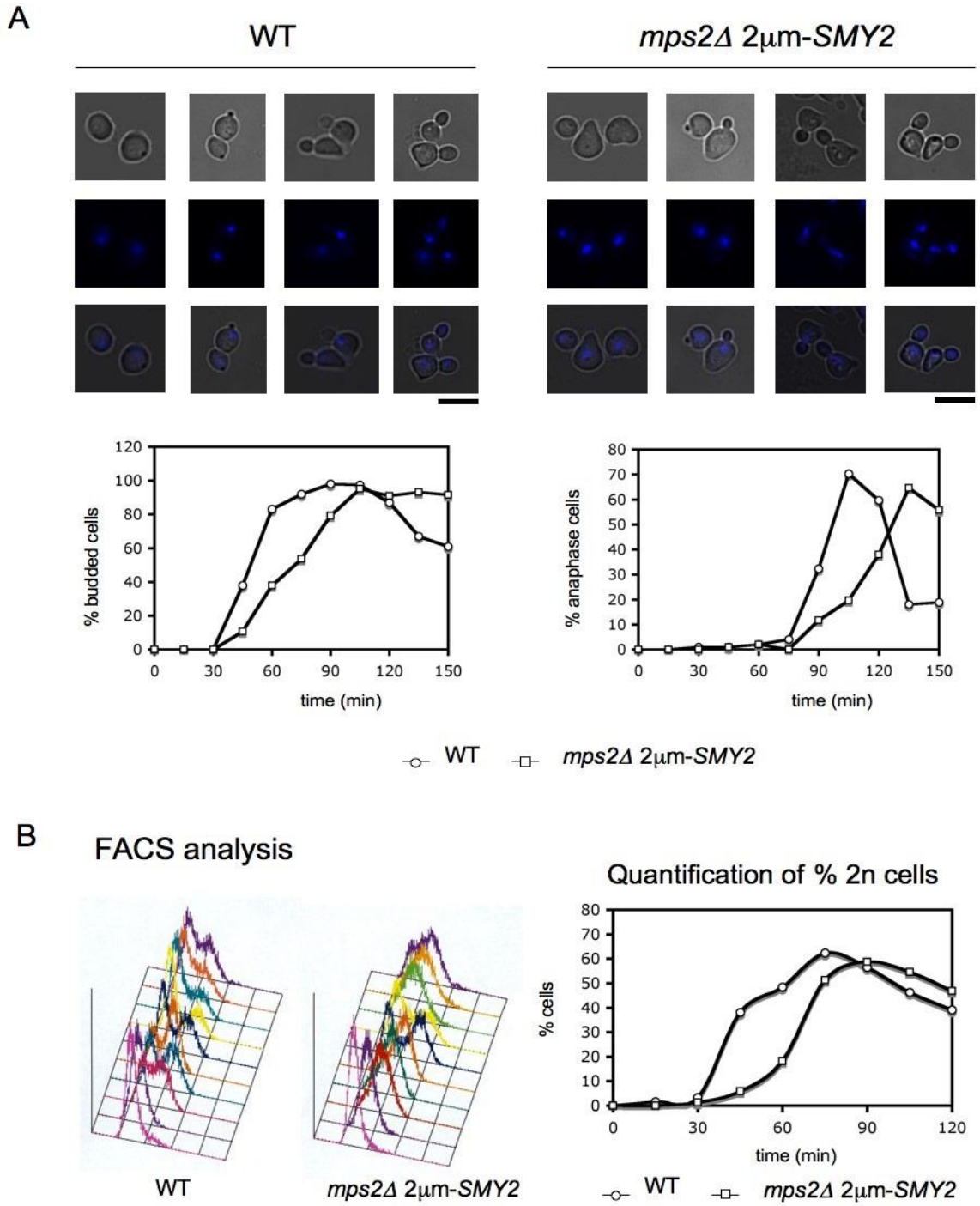

Figure 1: *mps2*∆ 2µm-*SMY2* cells show a general delay in cell cycle progression. Wild type and *mps2*∆ 2µm-*SMY2* cells were synchronized by α-factor and after release, samples were taken every 15 min. Each sample was analyzed by fluorescence microscopy after DAPI staining and FACS.

Our data indicate that the SESA network inhibits translation of the *POM34* mRNA in *mps2*Δ 2µm-*SMY2* cells. However, overexpression of *SMY2* in otherwise wild type cells was insufficient to down-regulate Pom34, suggesting that the SPB defect in *mps2*Δ cells induces the regulation of *POM34* mRNA (Figure 2).

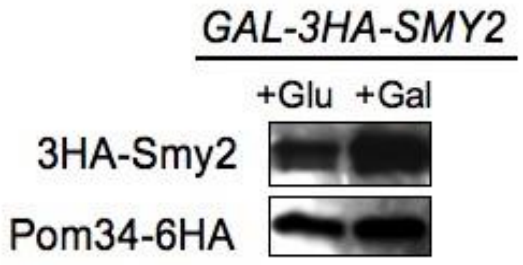

Figure 2: Overexpression of Gal1-*SMY2* does not induce depletion of Pom34. Immunoblotting of Gal1-*3HA-SMY2 POM34-6HA* cells. Cells were grown in raffinose medium at 30ºC. Glucose (+Glu) or glucose (+Gal) were added for 3 h at 30ºC. Cell extracts were analyzed with anti-HA antibodies.

On the other hand, we uncovered that translational repression of Pom34 by the SESA network is dependent on the growth medium of *mps2*Δ 2µm-*SMY2* cells. Pom34 protein level was significantly reduced in *mps2*Δ 2µm-*SMY2* cells with respect to wild type when grown in YPDA medium, but Pom34 level in *mps2*Δ 2µm-*SMY2* cells was similar to wild type levels when grown in SC-Leu medium (Figure 3).

pH of YPDA medium is 5.8, whereas pH of SC-Leu is 3.8. In order to rule out that lack of Pom34 suppression in SC-Leu growth media is due to this difference in pH, we adjusted the final pH value of SC-Leu to 5.8, and run a time-course experiment. *mps2*Δ 2µm-*SMY2* cells bred much rapidly in YPDA media compared to the SC-Leu grown cells (Figure 4a). Importantly, Pom34 protein accumulated in *mps2*Δ 2µm-*SMY2* cells grown in SC-Leu media, independent of the pH; in contrast *mps2*Δ 2µm-*SMY2* cells did not produce and detectable Pom34 protein in YPDA medium (Figure 4b). In another words, SESA is active YPDA grown cells, SESA is not active in cells grown in SC-Leu medium. Perhaps, translation machinery in yeast cells grown in YPDA differs from the cells grown in SC-Leu medium (Komili, 2007). Some other factor, another active pathway must be responsible for the bypass of *MPS2* deletion in SC-Leu. Perhaps the SESA network itself and its parallel networks are much more complicated than the current picture.

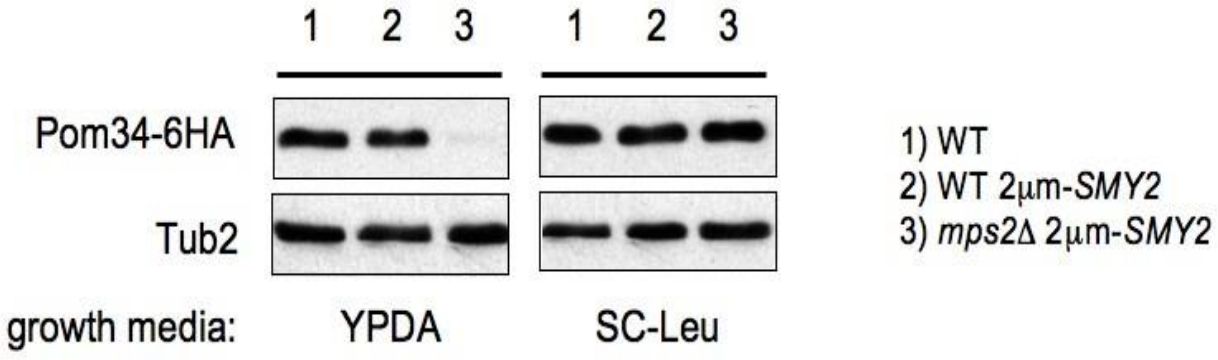

Figure 3: Translational repression of Pom34 in *mps2*Δ 2µm-*SMY2* cells is growth media dependent: Pom34 protein level is reduced in *mps2*Δ 2µm-*SMY2* cells grown in YPDA but not in SC-Leu. Yeast strains expressing *POM34*-6HA were grown either in YPDA or SC-Leu medium. Total cell extracts from these strains were analyzed by immunoblotting using anti-HA antibodies. Anti-Tub2 antibodies were used as loading control.

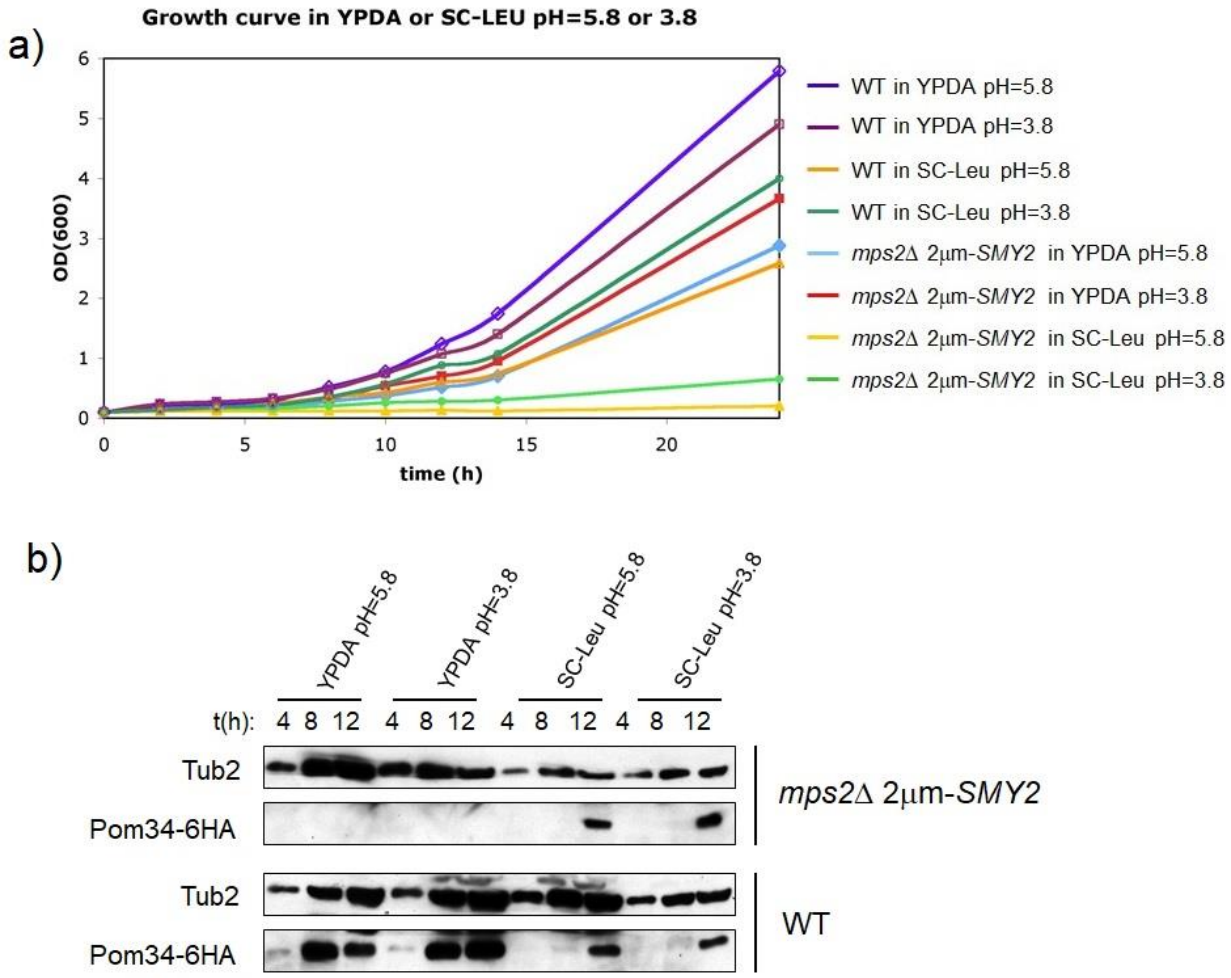

Figure 4: Growth rate and Pom34 levels in *mps2*Δ 2μm-*SMY2* cells is growth media dependent: Yeast strains expressing *POM34*-6HA were grown either in YPDA or SC-Leu medium. Total cell extracts taken from these strains at given time points were analyzed by immunoblotting using anti-HA antibodies. Anti-Tub2 antibodies were used as loading control.

CONCLUSION

In conclusion, SESA is active for YPDA grown cells, whereas SESA is not active in cells grown in SC-Leu medium. Thus, in cells grown in SC-Leu medium some other factor, another active pathway must be responsible for the bypass of *MPS2* deletion. Perhaps the SESA network itself and its parallel networks are much more complicated than the overall picture uncovered so far.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

BE designed and performed the experiments; and also wrote the manuscript.

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