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Overcoming Centrosome Duplication Defects by the SESA Network

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Abstract: 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, et al., 2009). Although many important points regarding SESA network have been discovered, many others remain obscure. In this study, we performed a genome-wide screen in order to unearth new members of the system and showed that Dhh1 is a member of the SESA network. Dhh1 is a known cytoplasmic DEAD-box helicase known to play role in translational regulation. Thus we propose that Dhh1 contributes to the highly selective nature of the inhibition of translation by SESA system.

Keywords: *S. cerevisiae*, Centrosome duplication, Translational control, SESA network

Introduction

In animal cells, the nuclear membrane disintegrates as they enter mitosis, recapitulates as they exit. In fungal cells such as *Saccharomyces cerevisiae*, that go through closed mitosis, the nuclear membrane remain 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). Many proteins are thought to be involved in the membrane-insertion process of SPB (Rose and Fink, 1987; Winey, et al., 1991; 1993; Spang, et al., 1995; Bullit E, et al., 1997; Adams and Kilmartin, 1999; Elliott, et al., 1999; Schramm, et al., 2000; Jaspersen, et al., 2002; Nishikawa, et al., 2003; Araki, et al., 2006). *MPS1* and *MPS2* (Monopolar Spindle) have been identified in a study of monopolar spindle cells (Winey, et al., 1991). Mps1 is a specific kinase, and its mutation causes cells to fail to complete cell cycle by single SPB. For this reason it most likely plays a role in the early stages of SPB duplication. *MPS2* encodes a membrane protein complexed with SPB protein Bbp1 (Schramm, et al., 2000). The *mps2-1* mutant cells have a small newly formed SPB structure that is attached to the former SPB via a bridge. However, this newly formed SPB is not located in the core membrane and is therefore devoid of nuclear microtubules (Winey, et al., 1991; Munoz-Centeno, et al., 1999). For this reason, it is suggested that the Bbp1-Mps2 complex is effective for the entry of the new SPB into the nuclear membrane (Schramm, et al., 2000). A similar phenotype has also been identified for cells with mutant *NDC1* (Winey, et al., 1993). Like Mps2, Ndc1 is a membrane protein and is found not only in SPB but also in the structure of nuclear pore complexes (Chial, et al., 1998). Another protein Nbp1 is required for the insertion of the newly formed SPB into the core membrane (Araki, et al., 2006). The *nbp1* mutant cells exhibit a 'dead' SPB phenotype, similar to the phenotype exhibited by the *mps2*, *ndc1*, *bbp1* mutants.

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MPS2 is an essential gene and encodes an integral membrane protein that directly interacts with Bbp1 and Nbp1 (Schramm, et al., 2000). We recently showed that the Smy2 can suppress the MPS2 essential function and that it cooperates with Eap1, Scp160, Asc1 during this task. We gave the name SESA (Smy2, Eap1, Scp160, Asc1) network to the system consisting of these four proteins (Sezen, et al., 2009). We have also shown that Bfr1 protein is involved in this system. *SMY2* is a gene whose product has completely unknown function (Higashio, et al., 2008; Georgiev, et al., 2007; Kofler, et al., 2005). *SCP160* encodes a protein that binds to mRNA (Li, et al., 2003; 2004). The gene product of *BFR1* is in contact with polysomes (Jackson, et al., 1994; Lang, et al., 2001). *ASC1* encodes a multifunctional protein, partially interacting with ribosomes (Baum, et al., 2004). Our previous studies have shown that Smy2, Eap1, Scp160, Bfr1 and Asc1 are present as a complex (Sezen, et al., 2009).

In detailed analysis, the SESA system was identified as part of a mechanism that regulates the translation event of the *POM34* mRNA. *POM34* encodes an integral membrane protein (Miao, et al., 2006), which together with the other two NPC components Pom152 and Ndc1, constitutes an important structure for NPC biogenesis. SESA does not affect the mRNAs of other NPC proteins (e.g., *POM152*, *NDC1*), and selectively suppresses the translation of only *POM34* mRNA. This indicates that SESA inhibits the translation of only a specific subset of mRNAs, not the start of the entire translation (Ergüden, 2017).

In cells where the SESA system is activated by an unknown mechanism upon SPB duplication defects, the *POM34* mRNA remains in the cytoplasm and translation is inhibited without binding to the polysomal rich endoplasmic reticulum. As a result, Pom 34 level falls to one fifth of the normal level. The diminished Pom34 level allows these cells to survive when the essential gene *MPS2* is deleted.

Our model suggests that the centrosome detects the imperfections in the insertion to the nuclear membrane and then this information is somehow transmitted to SESA. The reduction of translation of *POM34* mRNA, suppresses centrosomal duplication defects. The main goal of this work was to identify the other members of the SESA system. For this purpose, SESA active cells were transformed with the mTn3-lacZ / LEU2 Snyder yeast DNA library and new members of the SESA system were investigated.

Method

Transformation of *mps2*Δ pR316-*MPS2* Cells with Snyder Library

mTn3-*lacZ/LEU2* Snyder library DNA (Ross-Macdonald, et al., 1999) was digested with *NotI*. 50 ml overnight culture of *mps2*Δ pRS316-*MPS2* cells were spun down, washed with water and resuspended in 350 µL LiSorb. 50 µL carrier DNA was added and 50 µL yeast cells were transformed with 5 µL of digested library DNA. The tranformants were spun down, resuspended in 1 ml YPDA and recovered for 5 h at 23 C. After recovery the transformants are plated out on SC-Leu plates and incubated for 2-3 days so that each plate has around 200 isolated colonies. In the end the number of colonies must reach 3000-4000 colonies for each pool and 30,000 colonies in total.

The colonies were replica plated on SC+5-FOA plates and the DNA of the yeast strains which do not grow on SC+5-FOA plates were isolated. The point of insertion was identified by sequencing.

Strain Constructions and Growth Conditions

Gene deletions and epitope tagging of genes at their endogenous loci were performed using PCR-based methods (Janke, et al., 2004). All yeast strains were derivatives of S228c. Typically cells were grown in yeast extract peptone glucose medium (YPD) at 23°C.

Results and Discussion

The *mps2*Δ pRS316-*MPS2* pRS424-*SMY2* cells were transformed with the NotI enzyme-cut mTn3-lacZ / LEU2 library, and the colonies surviving on the LEU selective medium but inviable on the medium containing 5-FOA (5-fluoroorotic acid) were determined for subsequent analysis (Figure 1). All of the cells live on the LEU selective medium due to the plasmid pRS316-*MPS2*. Since this plasmid is lost on 5-FOA containing medium and genomic *MPS2* is deleted (*mps2*Δ), the survival of the cell depends on whether the SESA system works or not. The failure of a colony to survive in the medium containing 5-FOA is an indication that the SESA system is

not functioning. In the non-survivor cell, the random mutation of mTn3-lacZ / LEU2, has led to the deletion of a gene necessary for the SESA system to function. Determining which gene is deleted ensures the identification of possible new members of the SESA system.

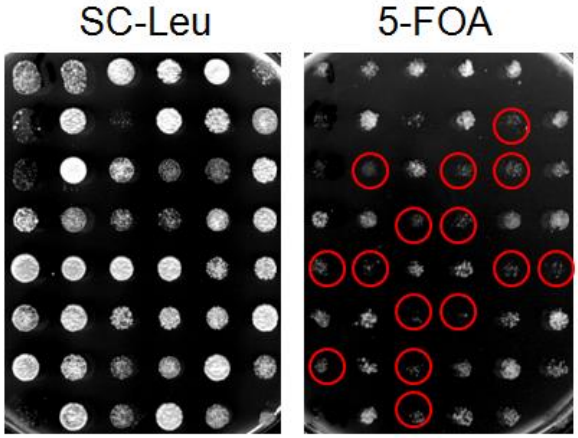

Figure 1. Identification of new members of the SESA network by means of deletion/insertion mutation using mTn3-*lacZ/LEU2* Snyder yeast DNA library. *mps2*Δ pRS316-*MPS2* cells were transformed with mTn3-*lacZ/LEU2* Snyder library DNA (Ross-Macdonald, et al., 1999) digested with *NotI* and the transformants are plated out on SC-Leu plates. The colonies were replica plated on SC+5-FOA plates and the DNA of the yeast strains which do not grow on SC+5-FOA plates were isolated. The point of insertion was identified by sequencing.

The DNA of the cells living in the LEU selective medium but inviable in the medium containing 5-FOA, upon transformation with the mTn3-lacZ / LEU2 library was isolated, cut with the *RsaI* enzyme, and amplified by PCR reaction. Some of the mutations obtained may be one of the known SESA members (*EAP1*, *SCP160*, *ASC1*, *BFR1*). To eliminate these results, the isolated DNA samples were tested by the colony PCR using the primers corresponding to the known SESA members. A result showing that all of the *EAP1*, *SCP160*, *ASC1*, *BFR1* genes are present in the colony PCR is an indication that another gene, which is a possible new SESA member and necessary for the operation of the SESA system, has been deleted from the cell. The DNA sequences of the remaining cells after this elimination were determined. From these data, *DHH1* was determined as a hit for the genome-wide screen.

DHH1 was deleted in the *mps2*Δ pRS425-*SMY2* cells and examined for any effect on the SESA system. As shown in Figure 2, the *mps2*Δ *dhh1*Δ pRS316-*MPS2* pRS425-*SMY2* cells do not grow on the medium containing 5-FOA when pRS316-*MPS2* plasmid is lost, loss of *DHH1* prevents the action of the SESA network. Thus Dhh1 is a possible SESA member.

Figure 2 Effect of *dhh1Δ* mutation on the SESA system. *mps2*Δ *dhh1*Δ pRS316-*MPS2* cells were transformed with the indicated plasmids and tested for growth on 5-FOA, at 23ºC.

Conclusion

We recently reported the presence of a network, named SESA, which allows cells to overcome SPB duplication failure (Sezen, et al., 2009). Despite the fact that 5 members of the SESA system (Smy2, Eap1, Scp160, Asc1, Bfr1) have been identified, the presence of other members is highly probable. In this study, it was aimed to determine the members of the SESA system by the application of a genome-wide deletion screen.

Dhh1 protein was identified as a new member of the SESA system upon these initial studies. It has been reported in the literature that Dhh1 is complexed with the SESA members Eap1 (Ash, et al., 2010) and Scp160 (Blewett and Goldstrohm, 2012) proteins. It was previously shown that SESA targets *POM34* mRNA at the translation stage rather than transcription or mRNA decay stages (Sezen B., et al., 2009). Accordingly, as the possible role of Dhh1 in the SESA system, it is suggested that it binds to *POM34* mRNA selectively and is involved in the transformation of *POM34* mRNA to the inactive sate.

This proposal is in accord with the previous findings that Dhh1 protein has a role in translational suppression (Carroll, et al., 2011). It has also been shown that during the early development of other eukaryotes such as *Xenopus* and *Drosophila*, Dhh1 orthologs in these organisms selectively suppress the translation of some mRNAs (Smillie and Sommerville, 2002; Nakamura, et al., 2001).

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