The SESA network links duplication of the yeast centrosome with the protein translation machinery

Bengü Sezen, Matthias Seedorf, and Elmar Schiebel[1]

Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), 69120 Heidelberg, Germany

The yeast spindle pole body (SPB), the functional equivalent of mammalian centrosome, duplicates in G1/S phase of the cell cycle and then becomes inserted into the nuclear envelope. Here we describe a link between SPB duplication and targeted translation control. When insertion of the newly formed SPB into the nuclear envelope fails, the SESA network comprising the GYF domain protein Smy2, the translation inhibitor Eap1, the mRNA-binding protein Scp160 and the Asc1 protein, specifically inhibits initiation of translation of *POM34* mRNA that encodes an integral membrane protein of the nuclear pore complex, while having no impact on other mRNAs. In response to SESA, *POM34* mRNA accumulates in the cytoplasm and is not targeted to the ER for cotranslational translocation of the protein. Reduced level of Pom34 is sufficient to restore viability of mutants with defects in SPB duplication. We suggest that the SESA network provides a mechanism by which cells can regulate the translation of specific mRNAs. This regulation is used to coordinate competing events in the nuclear envelope.

[*Keywords*: Regulation of translation; spindle pole body; nuclear pore complex; Smy2; Eap1; Scp160]

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The yeast spindle pole body (SPB) is the functional equivalent of the mammalian centrosome (Jaspersen and Winey 2004; Jaspersen and Stearns 2008). Centrosomes and SPBs duplicate once per cell cycle, organize microtubules, and are associated with cell cycle regulators (Pereira and Schiebel 2001; Stegmeier and Amon 2004). In addition, both are connected with the nuclear envelope. The budding yeast SPB is embedded in the nuclear envelope in a similar way to the nuclear pore complexes (NPCs). This incorporation into the membrane differs from the centrosome of higher eukaryotes that is anchored to the nuclear envelope via a proteinaceous link (Jaspersen and Winey 2004; Tzur et al. 2006).

The yeast SPB duplicates in G1/S phase of the cell cycle by a template-based mechanism (Adams and Kilmartin 1999; Jaspersen and Winey 2004). An intermediate in this duplication process, the duplication plaque, develops on the cytoplasmic side of the nuclear envelope as an extension of the pre-existing SPB. The duplication plaque then becomes inserted into the nuclear envelope as a consequence of the action of the essential *NDC1*, *MPS2*, *BBP1*, and *NBP1* genes (Winey et al. 1991, 1993; Adams and Kilmartin 1999; Schramm et al. 2000; Araki et al. 2006). A defect in these genes leads to a failure in SPB duplication such that the insertion of the duplication plaque into the nuclear envelope is either fully or partially compromised. The integral membrane protein Mps2 forms a tight complex with the cytoplasmic Bbp1. For SPB duplication the Mps2–Bbp1 complex cooperates with the Nbp1 protein, and the highly conserved integral membrane protein Ndc1 (Winey et al. 1993; Adams and Kilmartin 1999; Schramm et al. 2000; Araki et al. 2006; Mansfeld et al. 2006).

Ndc1 has an additional role in NPC biogenesis (Lau et al. 2004; Madrid et al. 2006; Mansfeld et al. 2006; Stavru et al. 2006). At the NPC, Ndc1 interacts with two other integral membrane proteins, Pom34 and Pom152 (Alber et al. 2007a,b; Onischenko et al. 2009). Ndc1, Pom34, and Pom152 have redundant functions in the biogenesis of NPCs (Madrid et al. 2006).

Curiously, *NDC1* shows genetic interactions with *EAP1* (Chial et al. 2000). In yeast, Eap1 and Caf20 are the two eIF4E-binding proteins that prevent formation of the eIF4E–eIF4G complex (Gingras et al. 1999), which is crucial for the recruitment of the 5′ end of mRNAs to the 40S ribosomal subunit during initiation of translation (Gingras et al. 1999). Eap1 and Caf20 inhibit general initiation of translation in response to stress conditions such as cadmium and diamides in the growth medium or the occurrence of membrane stress (Deloche et al. 2004; Mascarenhas et al. 2008).

SMY2 was discovered as a high dosage suppressor of the conditional lethal *myo2-66* mutation (Lillie and Brown

[1]**Corresponding author.**
E-MAIL e.schiebel@zmbh.uni-heidelberg.de; FAX 49-6221-545892.
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1994). Based on bioinformatics and in vitro studies with the truncated protein, it was concluded that the GYF domain of Smy2 interacts with proteins containing PPG repeats such as Eap1 and pre-mRNA splicing factors (Kofler et al. 2005; Georgiev et al. 2007). In addition, Smy2 has GYF domain-independent functions at COPII vesicles (Higashio et al. 2008). Thus, the molecular role of Smy2 is largely not understood.

We now describe a novel translational initiation control mechanism that is exerted by the Smy2–Eap1–Scp160–Asc1 network of proteins, named SESA, to regulate the initiation of translation of *POM34* mRNA, upon SPB duplication defects. The failure of the SPB to insert into the nuclear envelope triggers SESA to inhibit the translation initiation of *POM34* mRNA. The concomitant reduction in the level of Pom34 protein restores SPB duplication and so ensures survival of cells that encounter difficulties in inserting their SPB into the nuclear envelope.

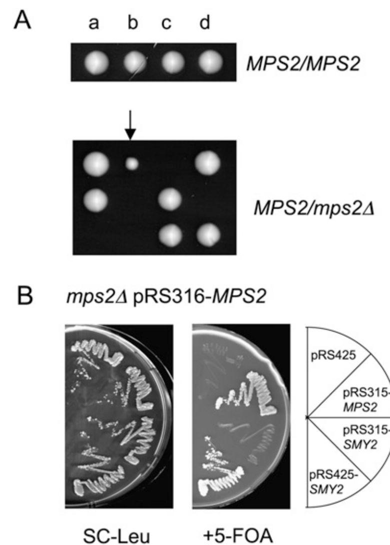

Figure 1. Suppressor screen for the lethal phenotype of *MPS2* deletion. (*A*) Spore analysis of *MPS2/MPS2* and *mps2Δ/MPS2* cells. a, b, c, and d indicate cells that developed from spores of one complete tetrad. The cells marked by an arrow did not contain the *MPS2* gene. (*B*) *SMY2* is a multicopy suppressor of the lethality of *mps2Δ* cells. *mps2Δ* pRS316-*MPS2* cells were transformed with the indicated plasmids. Transformants were grown on SC-Leu or 5-FOA plates for 3 d at 23°C.

Results

A genetic link between the SMY2 *gene and the SPB insertion machinery*

MPS2 encodes an essential, single membrane-spanning protein that inserts the newly formed SPB into the nuclear envelope (Winey et al. 1991; de la Cruz Munoz-Centeno et al. 1999; Schramm et al. 2000; Araki et al. 2006). Attempts to germinate *mps2Δ* spores from an *mps2Δ/MPS2* parent frequently led to the appearance of slow-growing *mps2Δ* survivors indicating that a suppressor mutation can ensure survival of cells lacking *MPS2* (Fig. 1A; arrow). A genetic screen for the wild-type genes that suppress the lethal growth defect of *mps2Δ* cells identified *SMY2* as a high gene dosage suppressor (Fig. 1B, sector 4). Smy2 is an endoplasmic reticulum (ER)-associated, GYF domain protein whose function remains to be elucidated (Kofler et al. 2005; Higashio et al. 2008).

SMY2 *functions through the translation inhibitor* EAP1

The cellular function of *SMY2* is obscured by reports on its interaction with proteins involved in translation control, pre-mRNA splicing, and protein secretion (Georgiev et al. 2007; Kofler et al. 2005; Higashio et al. 2008). To unravel the function of *SMY2*, we performed a synthetic lethal screen to reveal genes that become essential in the absence of *SMY2* (Supplemental Fig. S1; Supplemental Material). The screen identified two groups of genes: one set that is involved in the regulation of protein translation, and the other in membrane function (Supplemental Fig. S1C). This data suggested that *SMY2* may act as a functional bridge that connects these two processes.

A link between Smy2 protein and control of protein translation was provided by the report that the GYF domain of Smy2 has the ability to interact with the PPG repeats of the translation inhibitor Eap1 (Kofler et al. 2005). To determine whether *SMY2* functions through *EAP1*, we asked whether *EAP1* becomes essential for viability of *mps2Δ* 2μm-*SMY2* cells. Loss of *EAP1* was lethal for the *mps2Δ* 2μm-*SMY2* mutant cells (Fig. 2A, sector 3). In addition, the PPG mutant *eap1*[G624A] did not allow growth of *mps2Δ* cells (Supplemental Fig. S2C, row 8) and the GYF domain mutant *smy2*[Y234A] was no longer able to suppress the essential function of *MPS2* (Supplemental Fig. S2A, sector 3). Thus, the interaction between the GYF element of Smy2 and the PPG domain of Eap1 is essential for the bypass of *MPS2*. In contrast, *CAF20* that encodes the second eIF4E inhibitor (Ibrahimo et al. 2006), was not required for growth of *mps2Δ* 2μm-*SMY2* cells (Supplemental Table 1). This data suggests that *SMY2* functions through *EAP1*.

Coimmunoprecipitation experiments were performed to demonstrate that Smy2 interacts with Eap1 in vivo (Fig. 2B). The Eap1–Smy2 interaction was equally observed in wild-type and *mps2Δ* 2μm-*SMY2* cells (Fig. 2B), suggesting that the *mps2Δ* defect did not induce Smy2–Eap1 interaction. Moreover, the GYF domain of Smy2 was essential for the interaction, as Eap1 did not coimmunoprecipitate with the GYF domain-defective Smy2[Y234A] (Supplemental Fig. S2B). Together, this data suggests that Smy2 interacts in vivo with the translation inhibitor Eap1.

Eap1 allows bypass of MPS2 *function by inhibiting translation factor eIF4E*

Eap1 inhibits cap-dependent translation by competing with the initiation factor eIF4G (a subunit of the eIF4F cap-binding complex) for binding to eIF4E (Sonenberg and Gingras 1998). Because it was recently suggested that Eap1 may execute functions that are distinct from its role

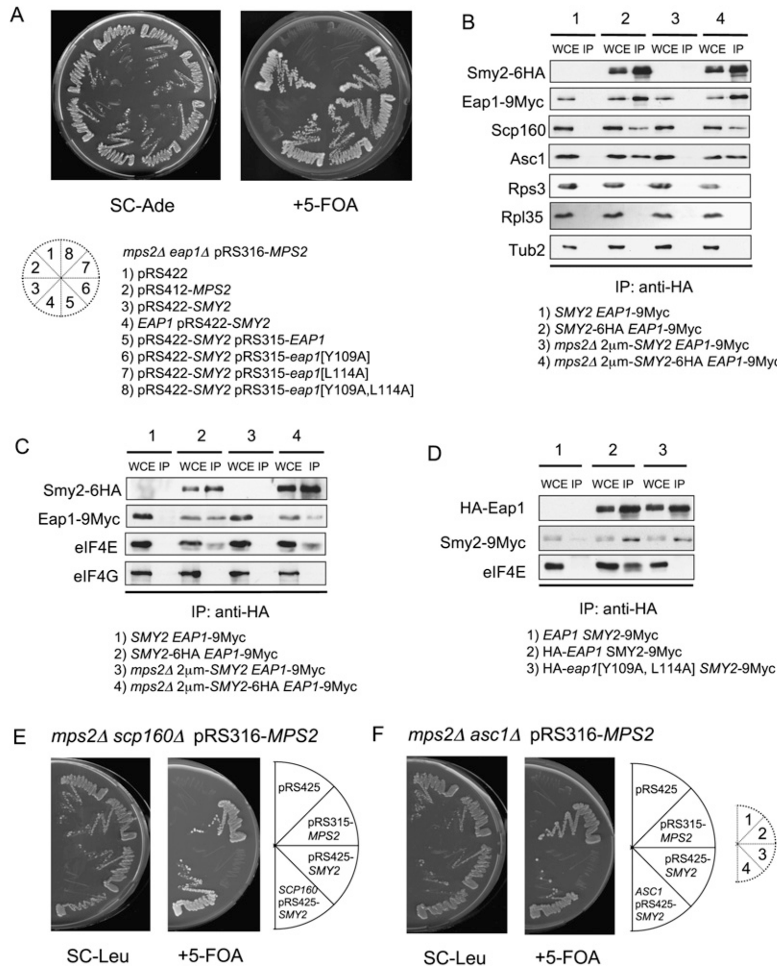

Figure 2. *EAP1*, *SCP160*, and *ASC1* are essential components of the *SMY2* pathway. (*A*) Dependency of suppression on *EAP1*. *mps2*Δ *eap1*Δ pRS316-*MPS2* cells were transformed with the listed plasmids and tested for growth on SC-Ade and 5-FOA plates. *eap1*[Y109A], *eap1*[L114A], and *eap1*[Y109A,L114A] code for Eap1 proteins with mutations that partially (*eap1*[Y109A], *eap1*[L114A]) or totally (*eap1*[Y109A,L114A]) disrupt binding to eIF4E (Fig. 2D; Ibrahimo et al. 2006). (*B*) Coimmunoprecipitation of Eap1, Scp160, and Asc1 by Smy2. Extracts from cells expressing *SMY2 EAP1*-9Myc or *SMY2*-6HA *EAP1*-9Myc were immunoprecipitated by anti-HA-coated magnetic beads and analyzed by immunoblotting with the indicated antibodies. (WCE) Whole-cell extract, 10% of the immunoprecipitation input; (IP) immunoprecipitation. (*C*) Coimmunoprecipitation of Eap1, eIF4E, and eIF4G by Smy2. Extracts from the yeast cells expressing *SMY2 EAP1*-9Myc or *SMY2*-6HA *EAP1*-9Myc were immunoprecipitated by anti-HA-coated magnetic beads and analyzed by immunoblotting with the indicated antibodies. Abbreviations as in *B*. (*D*) Coimmunoprecipitation of Smy2 and Eap1 and mutated Eap1 protein that fails to bind to eIF4E. Extracts from yeast cells with the indicated genotypes were immunoprecipitated by anti-HA-coated magnetic beads and analyzed by immunoblotting with anti-HA, anti-Myc, and anti-eIF4E antibodies. Abbreviations are as in *B*. (*E*,*F*) Dependency of suppression on *SCP160* and *ASC1*. *mps2*Δ *scp160*Δ pRS316-*MPS2* (*E*) or *mps2*Δ *asc1*Δ pRS316-*MPS2* cells (*F*) were transformed with the indicated plasmids and tested for growth on SC-Leu and 5-FOA at 23°C.

in the inhibition of translation (Chial et al. 2000), we asked whether *EAP1* regulates translation or plays some other function in *mps2*Δ cells. Coimmunoprecipitation experiments showed that eIF4E was associated with Smy2 and Eap1 in both wild-type and *mps2*Δ 2μm-*SMY2* cells, whereas eIF4G was excluded from this association (Fig. 2C). The latter was expected as Eap1 competes with eIF4G for the same site in eIF4E.

The interaction between Eap1 and eIF4E is impaired by the *eap1*[Y109A, L114A] mutations (Fig. 2D). This impairment of Eap1/eIF4E association relieves the inhibitory influence of Eap1 upon translation initiation (Ibrahimo et al. 2006). In this *eap1*[Y109A, L114A] background, *SMY2* was no longer able to suppress the lethal phenotype of the *mps2*Δ mutation (Fig. 2A, sector 8), even though the Eap1[Y109A, L114A] protein still associated with Smy2 in coimmunoprecipitation assays (Fig. 2D). Thus, inhibition of translation factor eIF4E by Eap1 binding is crucial for the viability of *mps2*Δ cells.

SCP160, ASC1, *and* BFR1 *become essential in cells lacking* MPS2

The proteins that regulate and cooperate with Eap1 in response to membrane defects are largely unknown (Deloche et al. 2004). With the *mps2*Δ 2μm-*SMY2* cells in hand, we were able to test genes for function in conjunction with *EAP1* and *SMY2*. We analyzed about 40 genes with roles in translation control, the UPR pathway (Sidrauski and Walter 1997), and nuclear envelope function (Supplemental Table 1) for their requirement in the viability of *mps2*Δ 2μm-*SMY2* cells. This analysis identified the genes coding for the polysome-associated, mRNA-binding protein Scp160 that has been shown to exist in a complex with Eap1 (Mendelsohn et al. 2003), the Scp160-interacting protein Asc1 (essential for ER localization of Scp160) (Baum et al. 2004), and the brefeldin A resistance protein Bfr1 (a component of polyribosome-associated mRNP complexes) (Lang et al. 2001), as being essential in the *mps2*Δ 2μm-*SMY2* background (Figs. 2E,F, 3A; Supplemental Table 1).

Coimmunoprecipitation experiments validated this genetic approach. Eap1, Scp160, and Asc1 were found to be in complex with Smy2 (Fig. 2B), and in addition, Scp160, Smy2, and Eap1 cofractionated in part in sucrose gradients (Supplemental Fig. S3). These interactions were not mediated by ribosomes, since the ribosomal proteins Rps3 (40 S ribosomal subunit) and Rpl35 (60 S ribosomal subunit) were not detected in the Smy2 immunoprecipitate (Fig. 2B). Interestingly, coimmunoprecipitation of

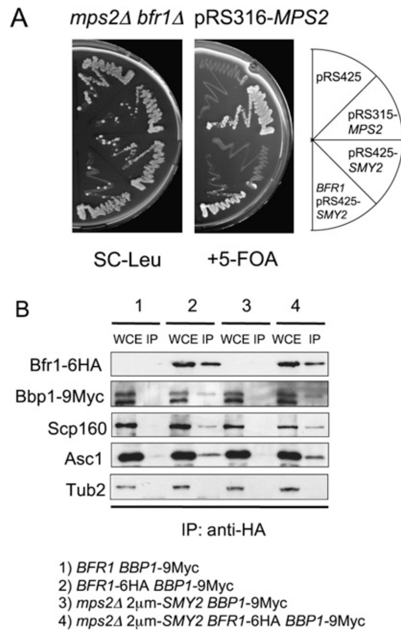

Figure 3. *BFR1* is essential for viability of cells lacking *MPS2*. (*A*) Dependency of suppression on *BFR1*. *mps2*Δ *bfr1*Δ pRS316-*MPS2* cells were transformed with the indicated plasmids and tested for growth on SC-Leu and 5-FOA plates at 23°C. (*B*) Coimmunoprecipitation of Bbp1 by Bfr1. Extracts from the yeast cells with the indicated genotypes were subjected to immunoprecipitation with anti-HA-coated magnetic beads and analyzed by immunoblotting with the indicated antibodies. Abbreviations as in Figure 2B.

Smy2 and Eap1 relied on the presence of the Scp160 protein but not on Asc1 (Supplemental Fig. S2D) and RNA, as RNaseA treatment only mildly affected the efficiency of coprecipitation (Fig. 4C). Thus, the Smy2–Eap1 interaction was stabilized by Scp160 but was not mediated by Asc1 and RNA.

Bfr1 coimmunoprecipitated with the Scp160 and Asc1 proteins, but failed to coimmunoprecipitate with Smy2 (Fig. 3B; Supplemental Fig. S4A), which establishes a distinct set of interactions from the Smy2–Eap1–Scp160–Asc1 association (Fig. 2B). In addition, we confirmed that the interaction of Bfr1 with Scp160 and Asc1 was RNA-dependent (Supplemental Fig. S4B), as has been reported for the Bfr1–Scp160 interaction (Lang et al. 2001). Thus, Bfr1 concurs with Scp160 and Asc1 in a manner that is distinct from the association of these proteins with Smy2. Based on the common genetic and biochemical interactions, we suggest that the gene products of *SMY2*, *EAP1*, *SCP160*, and *ASC1* closely cooperate in the suppression of *MPS2* deletion. This network of genes was named SESA. Bfr1 may interact through an RNA link with SESA network components.

Interestingly, two hybrid interactions between the SPB component Bbp1, which is in complex with Mps2, and the Bfr1 protein have been reported previously (Xue et al. 1996; Schramm et al. 2000). Coimmunoprecipitation experiments showed complexes containing Bfr1 and Bbp1 (Fig. 3B) and this interaction was independent of RNA (Supplemental Fig. S4B). Thus, Bfr1 and the SPB protein Bbp1 are present in common complexes. This Bbp1–Bfr1 interaction may provide the functional link to couple SPB duplication and the SESA network.

POM34 *mRNA is in a complex with Smy2*

In previous studies, Eap1 was identified as a global inhibitor of the initiation of protein translation in response to conditions that induce membrane stress. This effect of Eap1 could be measured in sucrose gradients by the increase in monosomes with concomitant decrease in

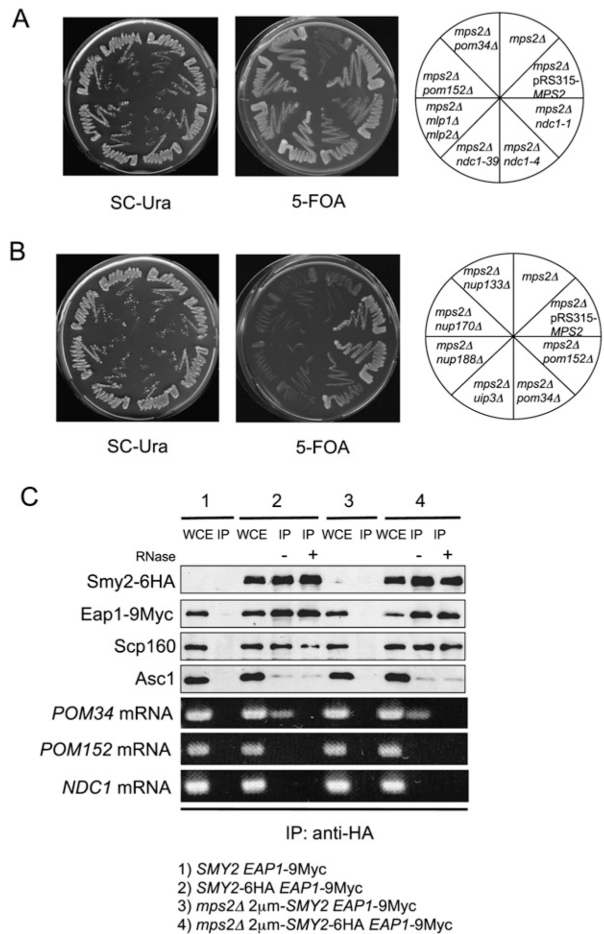

Figure 4. *POM34* mRNA binds to SESA components. (*A,B*) The indicated yeast cells were tested for growth on SC-Ura and 5-FOA plates at 23°C. All strains harbored initially the pRS316-*MPS2* plasmid. (*C*) Coimmunoprecipitation of *POM34* mRNA together with Eap1, Scp160, and Asc1 by Smy2. Extracts from yeast cells were incubated with anti-HA-coated magnetic beads, with or without RNaseA treatment, and analyzed by immunoblotting with the indicated antibodies. RNA was isolated from the immunoprecipitates and analyzed by RT–PCR using primers specific to *POM34*, *POM152*, and *NDC1*. In this immunoprecipitation experiment the efficiency of Asc1 coimmunoprecipitation was reduced probably because of the longer incubation time due to RNaseA treatment (cf. Figs. 2 and 4C). Abbreviations are as in Figure 2B.

polysomes upon stress conditions (Deloche et al. 2004). Similar sucrose gradient analysis of the ratio between isolated polysomes and monosomes in wild-type and *mps2*Δ 2μm-*SMY2* cells excluded the possibility of a global down-regulation of protein translation by Eap1 in *mps2*Δ 2μm-*SMY2* cells (Supplemental Figs. S5 [cytoplasmic fraction], S6 [membrane-bound fraction]).

We therefore tested whether the interaction of the mRNA-binding protein Scp160 with Smy2 and Eap1 could restrict translation inhibition to a specific subset of mRNAs, which may bind to SESA through the known mRNA-binding protein Scp160 (Frey et al. 2001). Attempts to identify mRNAs that were clearly regulated by Scp160 on the level of translation were unsuccessful. We thus turned to a genetic approach to identify mRNA candidates that are bound to Scp160.

We reasoned that both translation inhibition of an mRNA by the SESA network and loss of the targeted gene could suppress the essential function of *MPS2*. A candidate approach was therefore adopted to screen for genes whose inactivation would suppress the lethality of *mps2*Δ cells. It has been previously reported that the SPB duplication defect of *ndc1-1* cells is suppressed by the loss of the *POM152* gene that encodes an integral membrane protein of the NPC (Chial et al. 1998). Thus, our suppression analysis was initially focused on NPC components. *mps2*Δ lethality was suppressed by deletion of *POM34*, *POM152*, the double deletion of *MLP1* and *MLP2*, and conditional lethal *NDC1* alleles (Fig. 4A). Ndc1 is an integral membrane protein that has overlapping functions with Pom152 and Pom34 in NPC biogenesis (Chial et al. 1998; Tcheperegine et al. 1999; Madrid et al. 2006). The myosin like Mlp1 and Mlp2 are associated with the nuclear side of the NPC and have diverse roles in the retention of nonspliced mRNAs, nuclear transport, and SPB duplication (Strambio-de-Castillia et al. 1999; Galy et al. 2004; Niepel et al. 2005). The *mps2*Δ suppression phenomenon was restricted to these NPC components. Deletion of other nucleoporins (e.g., *NUP133*, *NUP170*, *NUP188*) or other integral membrane protein components of the nuclear envelope (e.g., *UIP3*) did not overcome the lethality of *mps2*Δ (Fig. 4B; see Supplemental Table I for the full list of genes tested).

As outlined above, the SESA network may bind and down-regulate the translation of target mRNAs to suppress the essential requirement of *MPS2*. To test this notion, we immunoprecipitated Smy2 and then isolated the mRNAs that were precipitated in a complex with Smy2. RT–PCRs performed on these RNA samples revealed that *POM34* mRNA associated with the SESA proteins, whereas *POM152* and *NDC1* mRNAs did not (Fig. 4C). Coimmunoprecipitation experiments performed in conjunction with UV cross-linking did not change these outcomes (data not shown). Other mRNAs tested and also found not to associate with Smy2 included *MLP1*, *MLP2*, *SEC61*, *ADH1*, and *SIC1* (data not shown). We further established that the association of *POM34* mRNA with Smy2 and Eap1 was dependent on the presence of Scp160 (Supplemental Fig. S7A,B). In contrast, disruption of *SMY2* or *EAP1* did not affect the association of *POM34* mRNA with Scp160 (Supplemental Fig. S7C). Taken together, these data indicate that *POM34* mRNA associates with the SESA network of proteins via an interaction with Scp160 protein.

SESA inhibits translation of POM34 *mRNA*

The presence of the translation initiation inhibitor Eap1 in the SESA network and the essential requirement of the Eap1–eIF4E interaction for *mps2*Δ suppression (Fig. 2A,D), prompted us to test whether SESA activity controls the translation of *POM34* mRNA. A prediction of this hypothesis is that Pom34 protein levels would be lower in *mps2*Δ 2μm-*SMY2* cells than in wild-type cells even though there would be no impact on *POM34* mRNA levels. To address this point, we first quantified the protein levels of NPC components in *mps2*Δ 2μm-*SMY2* and wild-type cells. Our analyses showed that Ndc1 levels were the same in both cell types, whereas Pom152 showed a modest decrease in *mps2*Δ 2μm-*SMY2* cells (Fig. 5A). Mlp1, Mlp2, Nup133, Nup170, and

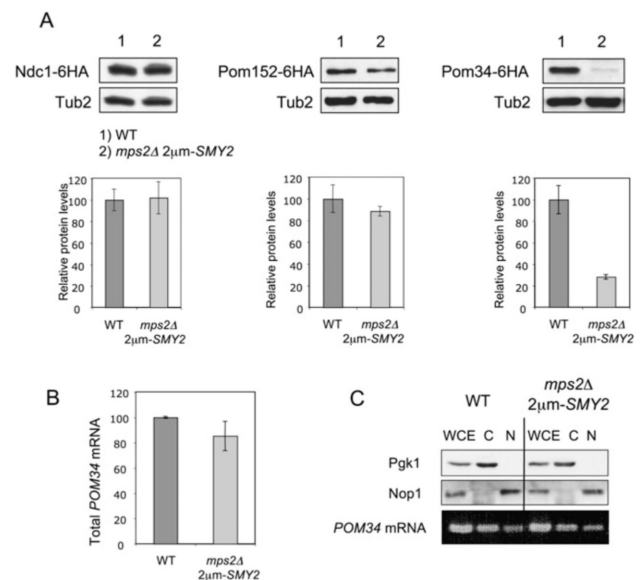

Figure 5. Strongly reduced amount of Pom34 in *mps2Δ* 2μm-*SMY2* cells. (*A*) Pom34 protein level is reduced in *mps2Δ* 2μm-*SMY2* cells. Total cell extracts from yeast strains expressing *NDC1*-6HA, *POM152*-6HA, or *POM34*-6HA were analyzed by immunoblotting using anti-HA antibodies. Anti-Tub2 antibodies were used as loading control. The graphs underneath the immunoblots show the quantification of three independent experiments, normalized for the wild-type protein levels. Bars are standard deviations around the mean value. (*B*) *POM34* mRNA levels are similar in wild-type and *mps2Δ* 2μm-*SMY2* cells. Total RNA extracts from wild-type (WT) and *mps2Δ* 2μm-*SMY2* cells were analyzed by quantitative RT–PCR using primers specific to *POM34* mRNA. (*C*) *mps2Δ* 2μm-*SMY2* cells do not show mRNA export defect. Wild-type and *mps2Δ* 2μm-*SMY2* cells were fractionated into nuclear and cytoplasmic fractions and analyzed by immunoblotting with anti-Pgk1 and anti-Nop1 antibodies. RNA was isolated from the nuclear and cytoplasmic fractions and analyzed by RT–PCR using primers specific to *POM34*.

Nup188 levels were also same in wild-type and *mps2Δ* 2μm-*SMY2* cells (data not shown). In contrast, the level of Pom34 protein was significantly lower in *mps2Δ* 2μm-*SMY2* cells than in wild type controls (Fig. 5A) even though the stability of the Pom34 protein assessed by the cycloheximide treatment was similar in both cell types (Supplemental Fig. S8). The modest decrease of Pom152 in *mps2Δ* 2μm-*SMY2* cells may arise from the strongly reduced level of Pom34 that interacts with Pom152 (Alber et al. 2007a,b).

The data described above suggest that Pom34 levels are decreased in *mps2Δ* 2μm-*SMY2* cells either because of a reduction in the efficiency with which *POM34* mRNA is translated, or because nuclear export of this mRNA is impaired or, finally, that the stability of *POM34* mRNA is reduced. Quantitative RT–PCR established that total levels of *POM34* mRNA were not significantly affected in *mps2Δ* 2μm-*SMY2* cells (Fig. 5B). Thus, we concluded that mRNA stability is probably not altered in *mps2Δ* 2μm-*SMY2* cells. Furthermore, a comparison of the levels of *POM34* mRNA in the cytoplasm of *mps2Δ* 2μm-*SMY2* and wild-type cells revealed no differences (Fig. 5C), indicating that the export of *POM34* mRNA was unperturbed by the *mps2Δ* 2μm-*SMY2* genotype. We conclude that *POM34* is likely regulated on the level of translation.

Membrane localization of POM34 *mRNA requires initiation of translation*

POM34 encodes an integral protein of the nuclear envelope (Miao et al. 2006). Its translation should therefore occur at the ER (Rapoport 1990). In order to pinpoint the step modulated by SESA control more precisely, we monitored the partitioning of *POM34* mRNA between cytosolic and membrane-bound fractions of yeast cell extracts alongside an assessment of the abundance of Scp160, Sec61, and Pgk1 proteins and ribosomal subunits.

In both wild-type and *mps2Δ* 2μm-*SMY2* cells, cytoplasmic Pgk1 was detected in the soluble cytoplasmic fraction and the integral membrane protein Sec61 (Deshaies et al. 1991) was found almost exclusively in the membrane fraction (Fig. 6A). Scp160 was enriched in the membrane-bound fraction, whereas the ribosomal subunit Rpl35 was more abundant in the cytoplasm. The latter distributions were as expected, since most ribosomes are present in cytoplasm while Scp160 is enriched with ribosomes at the ER (Supplemental Fig. S9A; Frey et al. 2001). Together, the cellular distributions of Scp160, Sec61, Pgk1, and Rpl35 proteins demonstrate that the fractionation into membrane and cytoplasmic fractions was successful.

According to RT–PCR data, the mRNAs of *SEC61* (Fig. 6A), *NDC1*, and *POM152* (Supplemental Fig. S6), which all encode integral membrane proteins, were slightly enriched in the membrane-bound fraction in both wild-type and *mps2Δ* 2μm-*SMY2* cells (Fig. 6A; Supplemental Fig. S6). In wild-type cells *POM34* mRNA distribution between the membrane fraction and the cytoplasm was similar to *SEC61* mRNA (Fig. 6A). Strikingly, however, in

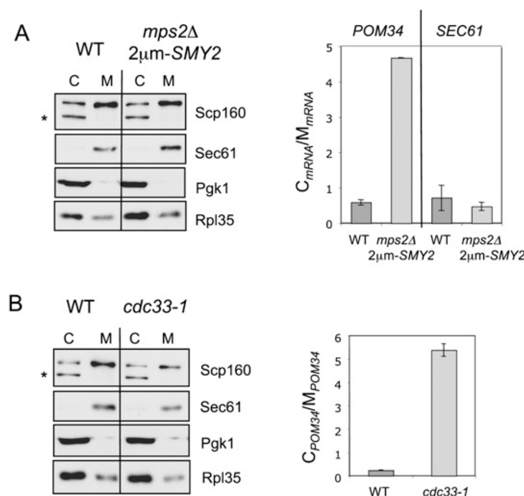

Figure 6. *POM34* mRNA accumulates in the cytoplasm in *mps2Δ* 2μm-*SMY2* cells. (*A*) Total cell extracts from wild-type and *mps2Δ* 2μm-*SMY2* cells were fractionated into cytosolic and membrane-bound fractions. Fractions were analyzed by immunoblotting using the indicated antibodies. The lower molecular weight band (asterisk) is a cytoplasmic degradation product of Scp160 (Frey et al. 2001). *POM34* and *SEC61* mRNA levels were determined by quantitative RT–PCR from three independent experiments. The graph shows the ratio of cytosolic *POM34* mRNA levels to the membrane-bound *POM34* mRNA levels. The bars indicate standard deviation of the results from the mean value. (C) Cytosolic fraction; (M) membrane-bound fraction. (*B*) Subcellular localization of *POM34* mRNA in the translation initiation mutant *cdc33-1*. Cells were grown in YPAD at 23°C and were shifted for 2 h to 37°C. The cellular fractionation and analysis was performed as in *A*.

mps2Δ 2μm-*SMY2* cells *POM34* mRNA was mostly found in the cytoplasm, and only very little in the membrane fraction (Fig. 6A). Thus, the cellular localization of *POM34* mRNA is significantly changed in *mps2Δ* 2μm-*SMY2* cells.

Mislocalization of *POM34* mRNA may arise from an inhibition of initiation of protein translation, which is required to target the mRNA in association with the nascent Pom34 protein to the ER membrane. If correct, a general inhibition of translation should result in the same cellular mislocalization of *POM34* mRNA. To test this prediction, we analyzed *POM34* mRNA localization in the conditional lethal translation initiation mutant *cdc33-1*, in which the function of yeast eIF4E is impaired (Brenner et al. 1988). *POM34* mRNA had a predominately cytoplasmic distribution in *cdc33-1* cells incubated at the restrictive temperature (Fig. 6B). The extent of the mislocalization of *POM34* mRNA in *cdc33-1* cells was only slightly higher than in *mps2Δ* 2μm-*SMY2* cells (Fig. 6A). This establishes that inhibition of translation initiation is indeed sufficient to mislocalize *POM34* mRNA to the cytoplasm.

We next asked whether translation elongation was also affected in *mps2Δ* 2μm-*SMY2* cells. Separating the translating membrane-bound ribosomal subunits and polysomes via a sucrose gradient fractionation was used as

experimental approach (Supplemental Fig. S6). Although less abundant in the membrane fraction, we observed no major difference in the relative *POM34* mRNA distribution between membrane-bound polysomes and monosomes in *mps2*Δ 2μm-*SMY2* cells with respect to wild-type cells (Supplemental Fig. S6C). Thus, our data do not suggest a defect in translation elongation for *POM34* mRNA in *mps2*Δ 2μm-*SMY2* cells. We also analyzed the cytoplasmic fraction of *POM34* mRNA in a sucrose gradient (Supplemental Fig. S5). This analysis showed that the cytoplasmic *POM34* mRNA, although more abundant in *mps2*Δ 2μm-*SMY2* cells than in wild-type cells (Fig. 6A), was predominately associated with the 40S, 60S, and 80S fractions of ribosomes in both cell types (Supplemental Fig. S5). A polysomal cytoplasmic fraction was not observed, which is consistent with the notion that translation elongation of *POM34* mRNA occurred at the ER. Thus, the main difference between wild type and *mps2*Δ 2μm-*SMY2* cells is the strongly reduced translation initiation efficiency of *POM34* mRNA in *mps2*Δ 2μm-*SMY2* cells. This defect then leads to the accumulation of *POM34* mRNA in the cytoplasm.

SPB duplication defects regulate Pom34 via the SESA network

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 (Supplemental Fig. S9B), suggesting that a defect in *mps2*Δ cells induces the regulation of *POM34* mRNA. The implication is that defects in SPB duplication may activate SESA. In this respect it is important to note that the SESA network component *EAP1* is essential for viability of the SPB duplication-defective *ndc1-1* cells that have normal NPCs (Chial et al. 2000). In addition, as was the case for *mps2*Δ (Fig. 1), the lethal growth defect of *ndc1-1* cells at 14°C was suppressed by the deletion of *POM34* (Fig. 7A), or high gene dosage of *SMY2* (Fig. 7B). Furthermore, Pom34 levels were reduced in *ndc1-1* 2μm-*SMY2* cells (Fig. 7C). These similarities between the consequences of mutations in *NDC1* and *MPS2* support the notion that defects in SPB duplication down-regulate *POM34* mRNA translation. Consistently, growth of the SPB duplication-defective *bbp1-1*, *mps2-42*, and *ndc1-4* cells at the restrictive temperature was rescued by *pom34*Δ (Fig. 7A; Supplemental Fig. S10A). Although, high gene copy 2μm-*SMY2* did not suppress the growth defect of these cells (data not shown), this is explained by the failure of *SMY2* suppression at elevated temperatures (Fig. 7D).

To further demonstrate that SESA becomes important for survival of mutants defective in SPB duplication, we analyzed the consequence of SESA inactivation in *bbp1-1* and *ndc1-4* cells. Deletion of SESA network components enhanced the growth defect of *bbp1-1* or *ndc1-4* cells grown at elevated growth temperatures (Fig. 7E; Supplemental Fig. S10B), suggesting that SESA network is important under conditions where *SMY2* is not overexpressed.

Down-regulation of POM34 *restores SPB duplication of* mps2 *cells*

SPB duplication fails in the absence of *BBP1*, *NDC1*, and *MPS2* function. In these mutant cells the duplication plaque is not inserted into the nuclear envelope. The nuclear side of the SPB with the component Spc110 is therefore not assembled, whereas the Spc42 becomes

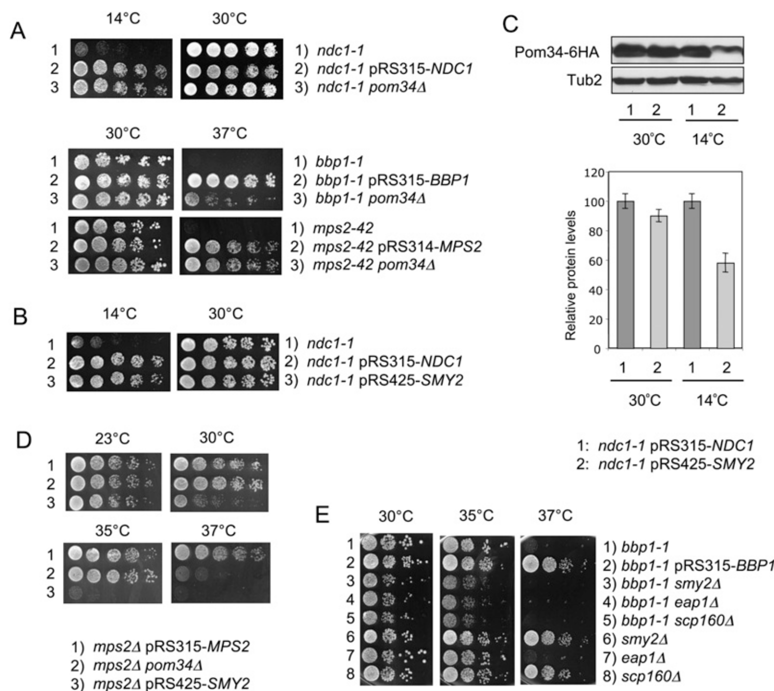

Figure 7. Genetic interactions between SESA and *POM34* with genes involved in SPB duplication. (*A*) Deletion of *POM34* suppresses *ndc1-1*, *bbp1-1*, and *mps2-42*. The listed yeast cells were tested for growth on YPAD plates at indicated temperatures. (*B*) *SMY2* is a multicopy suppressor of the cold-sensitive phenotype of *ndc1-1*. *ndc1-1* cells transformed with pRS315-*NDC1* or pRS425-*SMY2* were tested for growth on YPAD plates at 14°C and 30°C. (*C*) Pom34 protein level is reduced in *ndc1-1* 2μm-*SMY2* cells. Total cell extracts from yeast strains expressing *POM34*-6HA were analyzed by immunoblotting using anti-HA antibodies. Anti-Tub2 antibodies were used as loading control. The graph shows the quantification of three independent experiments, normalized for the wild-type protein levels. Bars are standard deviations around the mean value. (*D*) *mps2*Δ 2μm-*SMY2* and *mps2*Δ *pom34*Δ cells are temperature sensitive for growth. The listed yeast cells were tested for growth on YPAD plates at indicated temperatures. (*E*) Deletion of SESA components enhances *bbp1-1* growth defects. The listed yeast cells were tested for growth on YPAD plates at the indicated temperatures.

incorporated into the defective SPB (37°C: note *mps2-42* cells with only one Spc110-GFP signal but two Spc42-eqFP611 signals) (Fig. 8B; Schramm et al. 2000). However, deletion of *POM34* restored SPB duplication of *mps2-42* cells at 37°C indicating the importance of Pom34 levels in SPB duplication mutants (Fig. 8B; *mps2-42 pom34*Δ cells at 37°C with two colocalizing Spc110-GFP and Spc42-eqFP611 signals). Furthermore, analysis of *mps2*Δ 2μm-*SMY2* and *mps2*Δ *pom34*Δ cells at 23°C showed that the SPB duplicated in G1/S with similar kinetics as in wild-type cells (data not shown), and that the newly formed SPB carried the markers Ndc1-GFP, Bbp1-GFP, Nbp1-GFP, and Spc110-GFP (n = 150 cells) (Supplemental Figs. S11, S12). Thus, deletion or reduced translation of *POM34* suppresses SPB duplication defects.

Discussion

The initiation is the rate-limiting step in mRNA translation. Deregulating initiation by overexpression of the CAP-binding protein eIF4E leads to malignant transformation and therefore, not surprisingly, eIF4E is elevated in many human cancers (De Benedetti and Rhoads 1990; Lazaris-Karatzas et al. 1990). In addition, TOR signaling and stress situations including membrane defects inhibit global initiation of translation by regulating binding of proteins (4E-BPs) to the initiation factor eIF4E (Cosentino et al. 2000; Deloche et al. 2004; Matsuo et al. 2005; Ibrahimo et al. 2006).

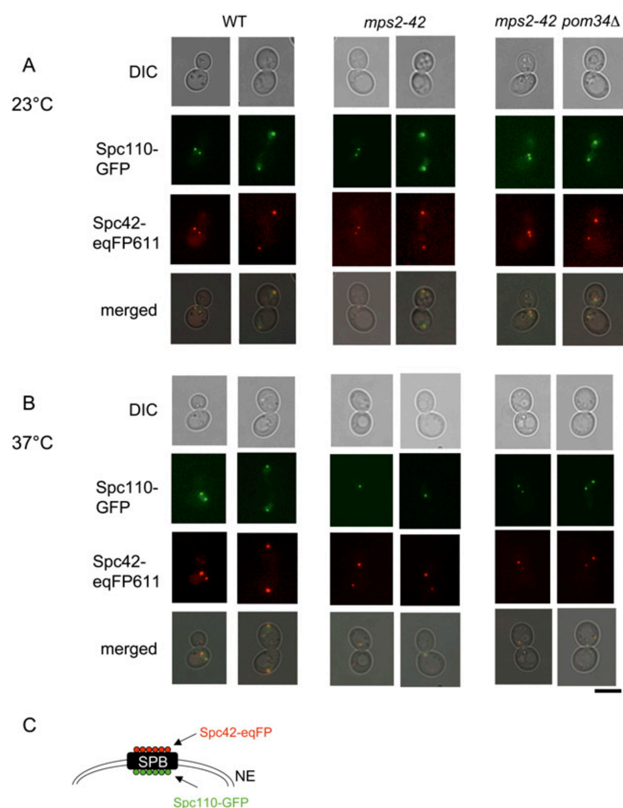

Figure 8. SPBs are inserted into the nuclear envelope in *mps2-42 pom34*Δ cells. (*A*,*B*) Analysis of wild-type, *mps2-42*, and *mps2-42 pom34*Δ cells with *SPC110*-GFP *SPC42*-eqFP611 by fluorescence and phase contrast (DIC) microscopy at 23°C (*A*) and 37°C (*B*) (2 h). Note that in *B* Spc110-GFP is only associated with one of the two Spc42-eqFP611-marked SPBs of *mps2-42* cells. This is the typical phenotype of cells with a defect in duplication plaque insertion (Schramm et al. 2000; Jaspersen and Winey 2004). Bars, 5 μm. (*C*) Shown is a cartoon of the SPB with the localization of Spc42 and Spc110 relative to the nuclear envelope (NE) (Adams and Kilmartin 1999).

In this study, we unraveled an unexpected link between the SPB duplication pathway and regulation of translation initiation of the *POM34* mRNA. In response to SPB duplication defects, the SESA network, comprising of the known mRNA-binding protein Scp160 (Frey et al. 2001; Li et al. 2003, 2004; Baum et al. 2004), the ribosome-associated Asc1 (Baum et al. 2004; Gerbasi et al. 2004), the translation inhibitor Eap1 (Cosentino et al. 2000), and the protein Smy2 (Kofler et al. 2005), was identified as being responsible for the translation control of *POM34* mRNA. We demonstrate that SESA inhibits translation of *POM34* mRNA by binding of the 4E-BP Eap1 to the conserved translation initiation factor eIF4E. By showing that Smy2, Eap1, Scp160, and Asc1 physically and functionally interact, we provide a first understanding of how Eap1 is regulated on the molecular level. This regulation of *POM34* mRNA by SESA is essential to ensure survival of cells with defects in SPB duplication.

SESA binds to POM34 *mRNA*

Based on the presence of the eIF4E inhibitor Eap1 in the SESA network, we expected to see a general inhibition of translation initiation in *mps2*Δ cells as is the case in cells exposed to membrane stress conditions (Deloche et al. 2004). Surprisingly, however, in *mps2*Δ 2μm-*SMY2* cells translation of proteins was not inhibited on a global scale (Supplemental Figs. S5, S6) indicating that the SESA network regulates only a subset of mRNAs.

The mRNA-binding protein Scp160 could target mRNAs to SESA regulation (Frey et al. 2001; Baum et al. 2004; Li et al. 2004). A microarray analyses of mRNAs released from affinity isolated Scp160-containing complexes identified a limited set of mRNAs that bind to Scp160 (Li et al. 2003). We tested the two most prominent mRNAs identified by Li et al. (2003), the *DHH1* and *YOR338w* transcripts, but did not find an enrichment of these mRNAs in anti-Smy2 immunoprecipitates nor any importance of the genes for survival of *mps2*Δ 2μm-*SMY2* cells (B Sezen, unpubl.). Furthermore, a recent study identified mainly mRNAs coding for proteins of the cell wall, plasma membrane, ER and nucleolus in association with Scp160 (Hogan et al. 2008).

To identify mRNAs that are regulated by SESA in the context of *MPS2* function, we turned to a genetic approach, which identified the *POM34* mRNA (Fig. 4) as being associated with SESA components. In addition, Pom34 levels were down-regulated in *mps2*Δ cells that require SESA for viability (Fig. 5A). Together, this strongly supports the notion that *POM34* mRNA is regulated by SESA.

SESA inhibits initiation of translation of POM34 *mRNA*

We suggest that SESA inhibits initiation of translation of *POM34* mRNA (Fig. 9, step 4). This model is supported by a number of findings. First, Eap1 blocks the crucial binding of eIF4G to eIF4E (Fig. 2C), which is normally an essential step in translation initiation (Gingras et al. 1999). Second, the Eap1[Y109A, L114A] mutations, which impair the Eap1–eIF4E interaction (Fig. 2D; Ibrahimo et al. 2006), also abrogate the function of SESA in *mps2*Δ cells (Fig. 2A) indicating that Eap1–eIF4E binding is essential for the down-regulation of Pom34. Third, although the *POM34* mRNA levels were unchanged in *mps2*Δ cells (Fig. 5B), Pom34 protein was strongly reduced in comparison with wild-type cells (Fig. 5A). A detailed analysis excluded *POM34* mRNA nuclear export defects and altered Pom34 protein stability as factors that could decrease Pom34 protein levels in *mps2*Δ cells (Fig. 5C; Supplemental Fig. S8). However, analysis of the distribution of *POM34* mRNA in *mps2*Δ cells clearly showed a shift toward the cytoplasmic fraction that has not initiated translation (Fig. 6A). A similar cytoplasmic shift of *POM34* mRNA was observed in cells defective in the translation initiation factor eIF4E (Fig. 6B; Brenner et al. 1988), supporting the idea that SESA inhibits initiation of translation. Forth, conditions that inhibited *POM34* translation did not affect translation of other mRNAs (*NDC1*, *POM152*, *MLP1*, *MLP2*, *SEC61*, *ADH1*, and *SIC1*). This implies that SESA is a specific inhibitor of translation of a subset of mRNAs. Smy2, Scp160, and Asc1 in the SESA network may confer Eap1 translation inhibition specificity toward a subset of mRNAs.

The Scp160 protein with its conserved KH RNA-binding domains (Frey et al. 2001) was deduced as the factor that binds the *POM34* mRNA in the SESA network (Fig. 4C; Supplemental Fig. S7). Scp160 shows genetic and biochemical interactions with the Asc1 protein, which is a core component of the 40S ribosomal subunit and as such binds Scp160 to ribosomes (Baum et al. 2004; Nilsson et al. 2004). However, also a cytoplasmic pool of Asc1 exists (Brodersen and Nissen 2005) that may direct the SESA–*POM34* mRNA complex to ribosomes. It is important to note that Asc1 functions as G-protein subunit coupled to glucose responsiveness in yeast (Zeller et al. 2007), and that the G protein α subunit Gpa1 transmits a signal through Scp160 (Guo et al. 2003). This raises the exciting possibility of a cross-talk between the SESA network and external stimuli.

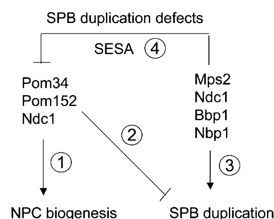

Figure 9. Model for the function of SESA network. (Step 1) Pom34, Pom152, and Ndc1 form a complex that functions in NPC biogenesis (Chial et al. 1998; Madrid et al. 2006; Alber et al. 2007a,b; Onischenko et al. 2009). (Step 3) Ndc1 has a dual role and together with Mps2, Bbp1, and Nbp1 it also functions in SPB duplication (Winey et al. 1993; Araki et al. 2006). (Step 2) Deletion of *POM34* or *POM152* or mutations in *ndc1* rescue SPB duplication defects (Fig. 4; Chial et al. 1998), suggesting an inhibitory role of the Pom34–Pom152–Ndc1 complex in SPB duplication. In response to SPB duplication defects, SESA down-regulates translation of *POM34* (Figs. 4–7). (Step 4) This in turn rescues the defect and allows for SPB duplication (Fig. 8; Supplemental Fig. S12).

Regulation of POM34 *mRNA in response to SPB duplication defects*

Mutants of *NDC1* and *MPS2* that failed to insert the SPB duplication plaque into the nuclear envelope showed genetic interactions with SESA components and, in addition, showed mislocalization of *POM34* mRNA and reduced levels of Pom34 in comparison with wild-type cells. These dependencies suggest a mechanism by which SESA becomes active in response to defects in SPB duplication (Figs. 1, 7; Chial et al. 2000; Schramm et al. 2000). This model is consistent with the activation of mammalian 4E-BPs by external stimuli (Gingras et al. 1999) or of yeast Eap1 by membrane defects (Deloche et al. 2004). In addition, elevated gene levels of *SMY2*, may arise due to chromosome missegregation in mutants with SPB duplication defects, which are genetically unstable (Winey et al. 1991, 1993; Schramm et al. 2000; Araki et al. 2006). Increased gene dosage of *SMY2* then likely makes the SESA network more sensitive to defects (Fig. 1).

The SESA network may be linked to the SPB by the Bfr1 protein and the interacting SPB component Bbp1 (Xue et al. 1996). Consistently, *BFR1* was found to be essential in cells lacking *MPS2* and the Bfr1 protein showed co-immunoprecipitation with Bbp1, Scp160, and Asc1 (Fig. 3). However, in contrast to SESA components, RNA mediated the interactions of Bfr1. Binding of Bfr1 to SESA-associated *POM34* mRNA could activate the ability of Eap1 to inhibit the translation of this mRNA.

Why is the NPC component Pom34 important for SPB duplication? Pom34, Ndc1, and Pom152 belong to a group of functionally redundant and interacting integral membrane proteins. They are part of the membrane ring of the NPC and as such important for NPC biogenesis (Fig. 9, step 1; Wozniak et al. 1994; Chial et al. 1998; Madrid et al. 2006; Miao et al. 2006; Alber et al. 2007a,b; Onischenko et al. 2009). A functional link between SPB insertion and NPCs, which are frequently observed near duplicating SPBs, was already proposed (Adams and Kilmartin 1999; Jaspersen and Winey 2004). Curiously, deletion of either *POM34* or *POM152* or mutations in *ndc1* suppress the essential function of *MPS2* (Fig. 4A). Based on this observation we suggest that the Pom34–Pom152–Ndc1 complex inhibits SPB duplication (Fig. 9, step 2). The Pom34–Pom152–Ndc1 complex either directly binds to SPBs and inhibits its duplication or SPBs and NPCs compete for components of a common nuclear envelope insertion machinery. The dual function of Ndc1 in SPB

duplication (Fig. 9, step 3) and NPC biogenesis (Fig. 9, step 1) supports the second competition model (Chial et al. 1998; Madrid et al. 2006). Defects in SPB duplication down-regulate expression of *POM34* via SESA (Fig. 9, step 4). Reduced Pom34 levels then relieve the inhibitory function of the Pom34–Pom152–Ndc1 complex. Thus, depending on the conditions, the SESA network may promote either SPB duplication or NPC biogenesis.

This study unraveled a novel mechanism by which cells can regulate the translation of specific mRNAs. In contrast to the general translation inhibition in response to, for example, membrane stress (Deloche et al. 2004), regulation by the SESA pathway enables the cell to modify the proteome in a very specific way at the level of translation. Thus, in respect of specificity SESA regulation is similar to the translation control of mRNAs containing a cytoplasmic polyadenylation element (CPE) by Maskin or 4E-T (4E-BP) in *Xenopus laevis.* In this case the CPE-binding protein (CPEB) acts as the specific RNA-binding protein (Stebbins-Boaz et al. 1999; Minshall et al. 2007). Another such example is the translational repression of *oskar* mRNA in *Drosophila* by Cup (a 4E-BP) where Bruno acts as the mRNA-binding protein (Nakamura et al. 2004; Chekulaeva et al. 2006; for reviews, see Richter and Sonenberg 2005; Sonenberg and Hinnebusch 2009).

Materials and methods

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). The strains and plasmids used in this study are listed in Supplemental Table 2. All yeast strains were derivatives of S228c with the exception of *ndc1-4* and *ndc1-39*, which were derived from W303 and were compared with the corresponding wild type.

Typically cells were grown in yeast extract peptone glucose medium (YPD) at 23°C. For analysis of temperature-sensitive mutants they were shifted for 2 h to 37°C before analysis.

Construction of CDC33, SMY2, *and* EAP1 *mutants*

SMY2 and *EAP1* with regulatory and coding regions were cloned into the *LEU2*-based yeast shuttle vector pRS425 and pRS315, respectively (Sikorski and Hieter 1989). Mutations in *SMY2* and *EAP1* were introduced by PCR-directed mutagenesis and confirmed by DNA sequencing. *cdc33-1* with regulatory and coding regions were cloned into the *LEU2*-based yeast integration vector pRS305 (Sikorski and Hieter 1989) after amplification by PCR from the genomic DNA of a *cdc33-1* strain (Brenner et al. 1988) and then inserted into our S288c strain background.

Antibodies and immunoblotting

Yeast extracts were prepared using alkaline lysis and TCA precipitation (Janke et al. 2004). To detect proteins by immunoblotting procedures, blocked membranes (Protean, Schleicher & Schuell) were incubated for 2 h at 20°C or overnight at 4°C with antibodies diluted in blocking buffer (PBS, 0.2% Tween 20, 5% dry milk powder) followed by peroxidase-conjugated secondary antibodies [Sigma] and detection with ECL (Roche Molecular Biochemicals). Anti-Scp160 (GST-Scp160), anti-Asc1 (NVIRVWQVMTAN-COOH), anti-Rps3 (VALISKKRKLVADC-CONH2), anti-Rpl35 (CPIRKYAIKV-COOH), anti-Sec61, anti-Clb2 (GST-Clb2$^{1-271}$), anti-Pds1 (GST-Pds1$^{1-173}$), and anti-Tub2 antibodies (yeast β-tubulin, GST-Tub2$^{436-457}$) were prepared in rabbits or sheep against purified recombinant proteins or peptides (Frey et al. 2001; Pereira and Schiebel 2003; Baum et al. 2004). Monoclonal mouse anti-Myc (9E10) and anti-HA (12CA5) antibodies were from Roche Molecular Biochemicals. Anti-Pgk1 and anti-Nop1 antibodies were gifts from M. Knop. Anti-eIF4E and anti-eIF4G antibodies were gifts from M. Ashe.

Coimmunoprecipitation

Logarithmically growing cells (3×10^8) were disrupted with glass beads in 300-μL immunoprecipitation buffer (50 mM triethanolamine, 150 mM KCl, 5 mM EDTA, 5 mM EGTA) containing a protease inhibitor cocktail (Boehringer Mannheim), phenylmethylsulfonyl fluoride (PMSF) and benzamidine. Lysates were incubated with 1% Triton X-100 for 10 min at 4°C. Extracts were cleared by centrifugation (6000 rpm for 10 min at 4°C). After removing an aliquot that served as the input control, the resulting extract was incubated with monoclonal anti-HA antibody, 12CA5, coated magnetic beads (Dynal) for 2 h at 4°C. Beads were washed three times with immunoprecipitation buffer containing 0.1% Triton X-100.

mRNA coimmunoprecipitation

Protein–mRNA coimmunoprecipitations were performed as described (Munchow et al. 1999; Bohl et al. 2000; Long et al. 2000). In brief, 3×10^8 logarithmically growing cells were disrupted with glass beads in 200-μL breakage buffer BB (50 mM HEPES-KOH at pH 7.3, 50 mM potassium acetate, 2 mM magnesium acetate, 1% Triton X-100) containing a protease inhibitor cocktail (Boehringer Mannheim) and 0.5% BSA. Extracts were cleared by centrifugation (6000 rpm for 10 min). After removing an aliquot that served as input control, the resulting extract was incubated at 4°C with monoclonal anti-HA antibody, 12CA5, coated magnetic beads (Dynal). Beads were washed three times with BB lacking BSA. Pellets were extracted with phenol-chloroform, ethanol precipitated, resuspended in RQ1 DNase buffer, and treated with RQ1 DNase (Promega). The remaining RNA was extracted, precipitated, and resuspended in water. RT–PCR was performed with 1 μL RNA as a template using the Qiagen RT–PCR kit and the conditions suggested by the manufacturer. The number of amplification cycles was adjusted to avoid reaching a plateau during PCR. For amplification of *POM34*, *POM152*, *SIC1*, and *ASH1* RNAs, we used 25 cycles, for amplification of *NDC1, MLP1,* and *MLP2* RNAs we used 24 cycles, whereas 22 cycles were used for amplification of *ADH1* RNA. The primers in Supplemental Table 3 were used for amplification.

RT-quantitative PCR

RNA was extracted from cells grown to logarithmic phase using Qiagen RNeasy kit following the manufacturer's protocol. A total of 4 μg of RNA was reverse transcribed using 7 μM oligo-dT and reverse transcriptase for 1 h at 37°C. The product was diluted 1:10 and used in the subsequent quantitative PCR reactions using *POM34* and *SEC61* primers in a Roche LightCycler using SYBR Green. Standard curves for each primer were generated using serial dilutions of yeast genomic DNA. Quantification of cDNA template concentrations was done using the standard curve for each primer.

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