

A Genetic Screen for High Copy Number Suppressors of the Synthetic Lethality Between *elg1Δ* and *srs2Δ* in Yeast

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ABSTRACT Elg1 and Srs2 are two proteins involved in maintaining genome stability in yeast. After DNA damage, the homotrimeric clamp PCNA, which provides stability and processivity to DNA polymerases and serves as a docking platform for DNA repair enzymes, undergoes modification by the ubiquitin-like molecule SUMO. PCNA SUMOylation helps recruit Srs2 and Elg1 to the replication fork. In the absence of Elg1, both SUMOylated PCNA and Srs2 accumulate at the chromatin fraction, indicating that Elg1 is required for removing SUMOylated PCNA and Srs2 from DNA. Despite this interaction, which suggests that the two proteins work together, double mutants *elg1Δ srs2Δ* have severely impaired growth as haploids and exhibit synergistic sensitivity to DNA damage and a synergistic increase in gene conversion. In addition, diploid *elg1Δ srs2Δ* double mutants are dead, which implies that an essential function in the cell requires at least one of the two gene products for survival. To gain information about this essential function, we have carried out a high copy number suppressor screen to search for genes that, when overexpressed, suppress the synthetic lethality between *elg1Δ* and *srs2Δ*. We report the identification of 36 such genes, which are enriched for functions related to DNA- and chromatin-binding, chromatin packaging and modification, and mRNA export from the nucleus.

Genome stability is of primary importance for the survival and proper functioning of all organisms. During DNA replication, the activity of DNA polymerases may be compromised by lesions or by the presence of secondary structures in the DNA. This may cause replication stalling and even fork collapse. Cells must react promptly to repair or bypass the DNA damage and to reactivate DNA replication. A number of mechanisms have been lately linked to this cellular response (Lee and Myung 2008).

PCNA is a homotrimeric ring that encircles the double-stranded DNA (Krishna *et al.* 1994) and plays a central role in DNA replication

by ensuring the processivity of the replicative DNA polymerases (Chilkova *et al.* 2007; Krishna *et al.* 1994). Many proteins interact with PCNA and use it as a platform that enables stable interactions with DNA. These proteins include factors involved in DNA replication, DNA repair, chromatin remodeling, and other DNA-related activities that are important for cell viability, cell division, and genomic stability (Warbrick 2000).

In response to DNA damage, PCNA can be mono-ubiquitinated at lysine 164 by the E2/E3 pair Rad6 and Rad18 (Hoege *et al.* 2002). Mono-ubiquitination of PCNA allows the binding of the translesion synthesis polymerases to PCNA, resulting in an error-prone repair mechanism (Acharya *et al.* 2008). Alternatively, PCNA can be further poly-ubiquitinated on the same lysine residue by a mechanism that also requires Ubc13-Mms2 (E2 heterodimer) and Rad5 (E3) (Ulrich and Jentsch 2000). This poly-ubiquitination coordinates an error-free repair mechanism, whose details are still unclear (Branzei *et al.* 2008). Interestingly, the same residue of PCNA (lysine 164) can be modified by the ubiquitin-like molecule SUMO. This modification takes place during S-phase or after high doses of DNA damage. An additional residue, lysine 127, can also be SUMOylated but not ubiquitinated. In contrast

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to mutations in lysine 164, those in lysine 127 do not lead to DNA damage sensitive phenotypes (Hoegge *et al.* 2002).

Two proteins have been shown to preferentially interact with SUMOylated PCNA in yeast: *Srs2* and *Elg1* (Armstrong *et al.* 2012; Kolesar *et al.* 2012; Parnas *et al.* 2010; Pfander *et al.* 2005). *Srs2* is a DNA helicase; depending on the assay used, *Srs2* has been shown to promote (Aylon *et al.* 2003) or prevent (Schiestl *et al.* 1990) homologous recombination. *Srs2* was found to be able to displace the Rad51 strand-exchange protein (homologous to bacterial RecA) from ssDNA (Krejci *et al.* 2003; Veaute *et al.* 2003). SUMOylated PCNA recruits *Srs2* to replication forks, where the helicase appears to prevent unscheduled recombination events (Papouli *et al.* 2005; Pfander *et al.* 2005).

The *Elg1* protein resembles the large subunit of Replication Factor C, a protein complex in charge of loading/unloading PCNA from DNA during replication. Mutations in *ELG1* lead to a variety of genomic instability phenotypes, including, among others, hyper-recombination, chromosome loss, elongated telomeres, and increased telomeric silencing (Banerjee and Myung 2004; Banerjee *et al.* 2007; Bellaoui *et al.* 2003; Ben-Aroya *et al.* 2003; Kanellis *et al.* 2003). Similarly to *Srs2*, mutations in *ELG1* suppress the sensitivity to DNA damaging agents of mutants of the post-replication repair pathway (Parnas *et al.* 2010). Both proteins are recruited to SUMOylated PCNA by a similar mechanism involving PCNA- (PIP) and SUMO-interacting motifs (Armstrong *et al.* 2012; Kolesar *et al.* 2012; Parnas *et al.* 2010; Pfander *et al.* 2005). Moreover, deletion of *ELG1* leads to accumulation of *Srs2* at the chromatin fraction, suggesting that *Elg1* may play a role in *Srs2* unloading, perhaps together with SUMOylated PCNA (Parnas *et al.* 2010). Remarkably, however, double deletion of the *ELG1* and *SRS2* genes results in a synthetic fitness reduction in haploid cells (Parnas *et al.* 2010) and in complete lethality in diploids (this study). These results imply that at least one of them must be active for viability. Because both proteins are active during DNA replication, it is likely that they provide alternative mechanisms to deal with specific DNA lesions or intermediates. However, alternative possibilities exist. Here we try to gain information about the function of these proteins by carrying out a genetic screen for genes that, when overexpressed, are able to rescue the synthetic lethality (SL) between *elg1* and *srs2* mutations.

MATERIALS AND METHODS

Yeast strains

Strain OP738 (MATa/MATalpha *elg1::KanMX/elg1:: KanMX srs2:: KanMX srs2:: KanMX ade2/ade2 ade3/ade3 leu2/leu2 ura3/ura3 his3/his3* [p1313: CEN-LEU2-ELG1-ADE3]) was used for the genetic screen. Strain MK166 (*lys2:: Ty1Sup ade2-1(o) can1-100(o) ura3-52 leu2-3, 112 his3del200 trp1del901 HIS3:: lys2:: ura3 his4:: TRP1:: his4*) (Liefshitz *et al.* 1995) and its derivatives were used for testing sensitivity to DNA-damaging agents and to measure recombination levels.

Genetic screen

A high copy number library in YE24 (a 2- μ m *URA3* plasmid) was used in the screen, carried out as described (Parnas *et al.* 2009). Each positive plasmid was extracted and retransformed into OP738. Only those that gave sectors were analyzed. Individual genes were subcloned into high copy number plasmids and retested.

GO (Gene Ontology) enrichment was calculated using an in-house score algorithm. The algorithm is a simple hypergeometric calculation; categories annotated with two few (≤ 3) or too many (≥ 500) genes were excluded from the enrichment calculation. Scores were corrected for multiple hypotheses testing an FDR threshold of 0.001.

RESULTS AND DISCUSSION

SL between *elg1* Δ and *srs2* Δ

Elg1 and *Srs2* physically interact with SUMOylated PCNA in a similar manner through their PIP and SUMO-interacting domains (Armstrong *et al.* 2012; Kolesar *et al.* 2012; Parnas *et al.* 2010; Pfander *et al.* 2005). In addition, *Elg1* seems to be required for the unloading of *Srs2* from chromatin (Parnas *et al.* 2010). We would thus expect mutations in *ELG1* and *SRS2* to show epistasis, such that the double mutant would not show a phenotype more severe than the single mutants. However, *elg1* Δ *srs2* Δ double mutants show, in comparison with each of the single mutants, increased sensitivity to DNA-damaging agents, such as the alkylating agent methylmethane sulfonate, the inhibitor of deoxynucleotides synthesis hydroxyurea, and the topoisomerase inhibitor camptothecin (Figure 1).

Mutations in each of the two genes cause an increase in homologous recombination (Ben-Aroya *et al.* 2003; Liefshitz *et al.* 1998). To characterize the type of recombination events that occur in the single and double mutants, we used strain MK166 (Liefshitz *et al.* 1995). This strain allows the detection of both gene conversion events between repeated sequences (Ty elements) or ectopic single sequences (*lys2* alleles) and direct-repeat recombination (DRR) at Ty elements and at the *HIS4* locus (Liefshitz *et al.* 1995). Whereas gene conversion transfers information between alleles, DRR results in the deletion of information located between the two repeats (Agmon *et al.* 2009). Table 1 shows that despite the fact that each mutant is hyperrecombinant, compared with the wt, they show a very different pattern of homologous recombination. Whereas *srs2* Δ shows increased levels of gene conversion events, the *elg1* Δ mutant shows both high gene conversion and high DRR levels. The *elg1* Δ *srs2* Δ double mutant shows the same level of DRR as *elg1* Δ ; however, it exhibits a synergistic effect for gene conversion: *lys2* gene conversion is elevated eightfold, and the level of Ty ectopic conversion is increased 80-fold above that of the wt strain (Table 1). We interpret these results as follows: The activity of *Srs2* prevents gene conversion events, probably by evicting the Rad51 recombination protein from the DNA (Krejci *et al.* 2003; Veaute *et al.* 2003). In the absence of *Srs2*, we see an increase in gene

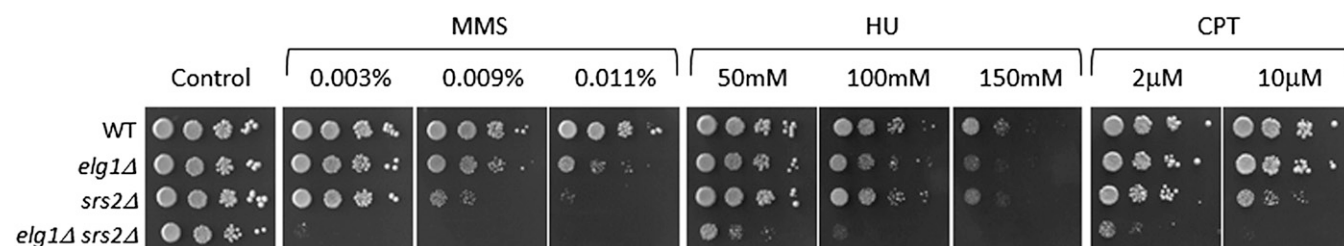


Figure 1 The *elg1* Δ and *srs2* Δ mutants display synergistic sensitivity to DNA-damaging agents. Ten-fold serial dilutions of logarithmic cultures were plated on SD-complete plates containing either methylmethane sulfonate (MMS), hydroxyurea (HU), or camptothecin (CPT) at the concentrations shown.

■ **Table 1 Recombination frequency**

Strain	His ⁺ (DRR)	Ty DRR	Ty GC	Lys ⁺ (GC)
wt	1 (10×10^{-6})	1 (1×10^{-6})	1 (0.5×10^{-6})	1 (0.25×10^{-6})
<i>srs2Δ</i>	1.1	1.1	3.5	2
<i>elg1Δ</i>	4.5	12	5	1.5
<i>elg1Δ srs2Δ</i>	4.4	11	80	8

Direct repeat recombination (DRR) is measured between tandem repeats at the *HIS4* locus or between Ty elements. Gene conversion (GC) measures the transfer of information between Ty elements or two copies of the *LYS2* gene carrying different mutations.

conversion events but not in DRR events, which are probably carried out by single strand annealing and do not require Rad51 (Krejci *et al.* 2003). In contrast, the absence of Elg1 promotes all types of recombination events, either because Elg1 does not allow the first steps of recombination to take place, or, as has been suggested (Davidson *et al.* 2012), because in its absence spontaneous DNA damage accumulates. The results of the double mutant, however, suggest that Elg1 activity restricts the level of gene conversion events of *srs2* mutants, and thus both proteins must play some role during the actual recombination process.

elg1Δ srs2Δ double mutants are extremely sick as haploids (Parnas *et al.* 2010), indicating that cells need at least one of the proteins to carry out a function important for fitness, probably some type of repair that is so common that it occurs often during DNA replication, such as fork stalling response. Previous work showed that many of the phenotypes of *srs2Δ* are stronger in diploids, and that several mutations affecting DNA repair produce a synthetic sick phenotype when combined with *srs2Δ* as haploids, but are completely lethal as diploids (Klein 2001). We thus crossed an *elg1Δ srs2Δ* haploid with either an *elg1Δ* haploid, an *srs2Δ* haploid or a double mutant *elg1Δ srs2Δ* of the opposite sex. Mating took place at normal frequencies. Zygotes were micromanipulated under the microscope to fixed positions in the plate and were then incubated for 48 hr. Thirty-two of 32 zygotes and 31 of 32 zygotes were able to form colonies in the first two crosses (with single *elg1Δ* and *srs2Δ* mutants, respectively), whereas only 2 of the 80 *elg1/elg1 srs2/srs2* zygotes formed viable colonies after micromanipulation. Microscopic observation of the zygotes showed that the majority produced between 0 and 2 cell divisions before dying. Thus, diploid *elg1/elg1 srs2/srs2* strains are inviable.

A high copy number screen for suppressors of the SL between *elg1Δ* and *srs2Δ*

Our high copy number screen was based on a red/white colony phenotype (Koren *et al.* 2003; Koshland *et al.* 1985; Parnas *et al.* 2009). *ade2* mutants form red colonies due to the accumulation of a red pigment. Mutations in the upstream-acting gene *ADE3* prevent the accumulation of the red pigment and render the cells white. An *ade2 ade3 elg1Δ srs2Δ* diploid strain (OP738) kept alive by the presence of a *LEU2*-marked plasmid carrying the *ELG1* gene as well as the *ADE3* gene forms uniformly red colonies (as the *ade3* mutation is complemented, and any cell that loses the plasmid dies; Figure 2). These cells were then transformed with a yeast genomic library cloned in a high copy number plasmid (containing the *URA3* marker). Cells that received a plasmid with a gene that, when overexpressed, can suppress the SL phenotype of *elg1Δ srs2Δ*, are now able to lose the *ELG1-ADE3-LEU2* plasmid, and therefore show white/red sector colonies (Figures 2 and 3). The sectoring pattern (number and size of sectors) reflects the rate of viable plasmid loss, and thus provides a measure of the efficiency of suppression. Examples of such patterns are seen in Figure 3.

After screening ~50,000 yeast transformants, 92 candidates showed some level of colony sectoring. Plasmids (each containing a ~8 kb

insert on average) were obtained from these colonies and retransformed into OP738. Only 54 of these candidates clearly exhibited sectors upon retransformation. These plasmids were sequenced to determine the genomic region responsible for the suppression. Many plasmids carried overlapping genomic fragments, and in total were found to represent 34 genomic regions. Subcloning experiments were then carried out to identify the genes responsible for the suppressive phenotype. With a few exceptions (*RTT103* and *NSE3*; *CHD1* and *PAB1*), only one gene per plasmid caused increased white sectoring. Table 2 shows the names and functions of the 36 genes identified.

The genes identified span a large number of functions, pathways and cellular location. Analysis of GO (gene ontology) term enrichment (Table 3) shows that this gene set is enriched for genes of chromosomal location, with functions related to DNA- and chromatin-binding and involved in biological processes related to DNA packaging and modification. Additionally, there is an enrichment for genes involved in regulatory mechanisms as well as genes that participate in the response to DNA damage. Below, we present the genes, divided into functional categories obtained by reading the literature regarding each of the genes. Naturally, this division is artificial, as many of the categories overlap, and the precise mechanism of suppression is still unknown.

Genes affecting genome stability: This category includes the following genes:

ECO1/CTF7: *Eco1* is a protein essential for sister chromatid cohesion (Skibbens *et al.* 1999; Toth *et al.* 1999). It has an acetyltransferase activity that acetylates the cohesin subunit *Smc3*. This acetylation has been shown to be critical for the establishment of cohesion during S phase (Unal *et al.* 2008). *Eco1* interacts with *PCNA* through a PIP motif (Moldovan *et al.* 2006), thus effectively coordinating between cohesion establishment and DNA replication. It was found that loss of *PCNA* SUMOylation by deletion of the SUMO ligase *SIZ1* rescued the temperature sensitivity of *eco1* mutants, indicating a role for SUMO in this process (Moldovan *et al.* 2006). Elg1 has been shown to affect sister chromatid cohesion (Parnas *et al.* 2009) and genetic antagonistic interactions have been observed between Elg1 and *Eco1*: deletion of *ELG1* partially rescues the phenotypes of a temperature sensitive *eco1* mutant, whereas its overexpression enhances its phenotypes (Maradeo and Skibbens 2009; Maradeo and Skibbens 2010). Despite their common interaction with *PCNA*, no physical or genetic interactions have been detected between *Eco1* and *Srs2*.

NSE3: *Nse3* is part of the *Smc5-6* complex, which is structurally similar to cohesin, but whose precise function still remains enigmatic (Bustard *et al.* 2012). This complex was found to be required for a variety of DNA repair activities, including sister chromatid recombination, and mutants defective for its components exhibit an unusual amount of recombination intermediates (Bustard *et al.* 2012), a phenotype shared with both *elg1Δ* and *srs2Δ* mutants. Interestingly, the complex contains a SUMO-ligase and potentially a ubiquitin-ligase. A recent publication links cohesin SUMOylation by the *Smc5-6* complex

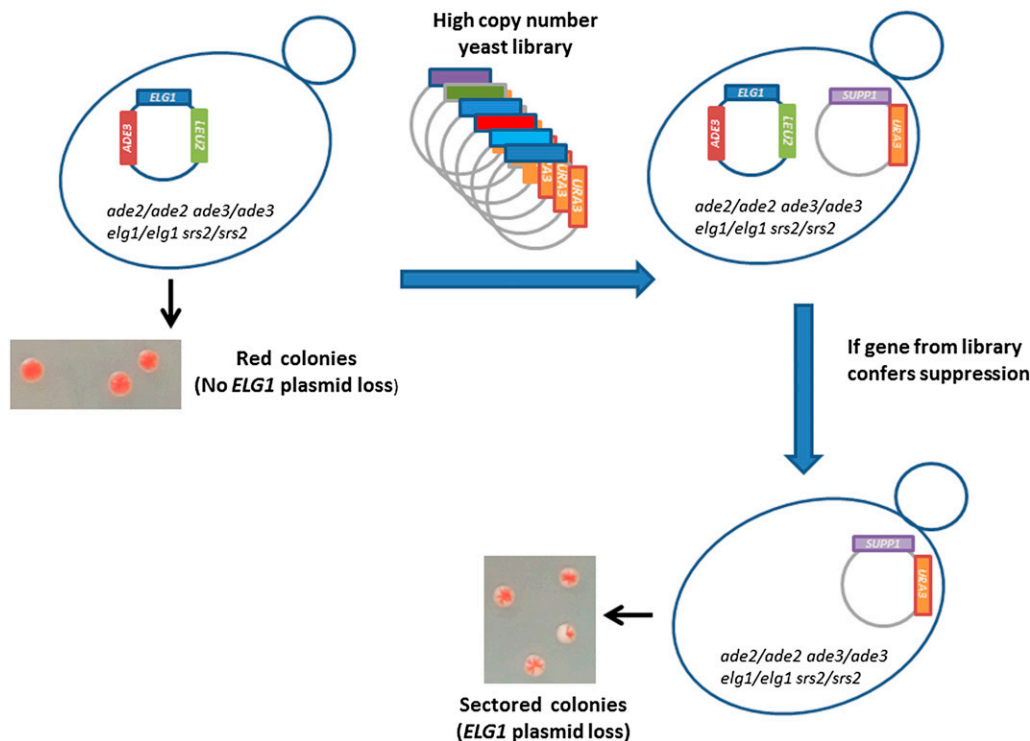


Figure 2 Schematic representation of the screen. A diploid double mutant *elg1Δ srs2Δ* strain is kept alive by the presence of a plasmid carrying the *ELG1* gene. The *ADE3* marker on the plasmid confers a red pigment to the cells carrying it (as the strain is *ade2 ade3*). Since any cell that loses the plasmid during colony formation dies, all colonies are uniformly red. This strain was transformed with a high copy number library carrying random fragments of the yeast genome. Cells that received a plasmid that suppresses the *SL* phenotype (*SUPP1*) can now lose the *ELG1*-containing plasmid, becoming white. These cells create white or red/white sector colonies.

to cohesion establishment, in an *Eco1*-independent fashion (Almedawar *et al.* 2012).

***MPH1*:** *Mph1* is the yeast homolog of FANCM, a human gene that, when mutated, leads to Fanconi anemia. *Mph1* is a DNA helicase involved in error-free bypass of DNA lesions. It has been proposed that *Mph1* may promote sister chromatid recombination as a way of bypassing lesions that stall fork progression (Ede *et al.* 2011). Interestingly, deletion of *MPH1* rescues the DNA damage sensitivity of mutants of the *Smc5-6* complex (Chavez *et al.* 2011; Chen *et al.* 2009), whereas *Mph1* overexpression exacerbates the mutants' phenotypes (Chen *et al.* 2009). Physical and negative genetic interactions have been observed between *Mph1* and *Srs2* (Chiolo *et al.* 2005; St Onge *et al.* 2007). *mph1* mutants share with both *elg1* and *srs2* genome instability phenotypes (Daee *et al.* 2012), suggesting that *Mph1* may be sharing repair functions with both genes.

***DDC1*:** *Ddc1* forms part of a PCNA-like molecule (called the 9-1-1 clamp). Loading of this heterotrimeric ring onto DNA is essential for the activation of the DNA damage response (Melo *et al.* 2001). This clamp has been recently proposed to play a pivotal role in an error-free lesion bypassing mechanism that operates in parallel to *Srs2* (Karras *et al.* 2013).

These four genes seem to be exerting their suppressive effect by affecting processes that take place during DNA replication, at the fork. Their overexpression may prevent stalling or may be able to act in DNA repair, either replacing a common function of *Elg1* and *Srs2*, or providing alternative repair mechanisms. *Ddc1* overexpression may lead to a stronger or extended DNA checkpoint response, which may help cope with fork stalling. Alternatively, the lethality between *elg1* and *srs2* may be due to lesions left behind the fork in the absence of the two repair activities, and overexpression of proteins that participate in alternative repair mechanisms may be able to repair this lethal damage, rescuing the cells.

Genes affecting histones and chromatin: This category includes the following genes:

***HTB1*:** DNA is packaged in eukaryotic cells around nucleosomes composed of two units each of the H2A-H2B and H3-H4 heterodimers. *HTB1* encodes a copy of histone H2B. Ubiquitination of this histone on residue K123 is a prerequisite for histone H3 modification and is essential for a variety of cellular processes, including gene expression and DNA repair (Dover *et al.* 2002; Robzyk *et al.* 2000).

***RTT106*:** During DNA replication, nucleosomes ahead of the replication fork are disassembled, and the recently replicated DNA is reassembled into nucleosomes using both the old, parental histones, as well as newly synthesized histones. Deposition of newly synthesized H3-H4 requires histone chaperones, including *Rtt106* (Li *et al.* 2012). The dimeric histones delivered by *Rtt106* are marked by acetylation at lysine 56 of histone H3 (Su *et al.* 2012).

***RTT109*:** *Rtt109* is a histone acetyltransferase that is required for proper acetylation of histone H3 at lysine 56 (H3K56), which occurs during S phase and DNA damage repair. The formation of H3K56ac

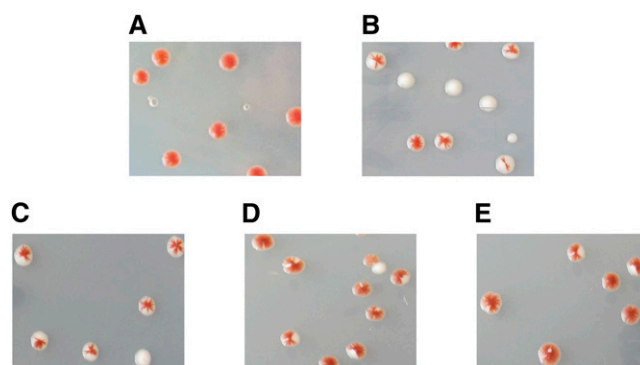


Figure 3 Examples of sectoring phenotypes observed in the presence of various high copy number plasmids. (A) Empty vector. (B) *ELG1*. (C) *MOB1* (strong suppressor). (D) *RTT109* (medium). (E) *PAB1* (weak suppressor).

■ Table 2 Genes in the screen

Gene Name	Short Description	Degree of Suppression
BAP2	High-affinity branched aminoacid permease	Strong
ECO1	Acetyltransferase required for Smc3p acetylation during the establishment of sister chromatid cohesion	Strong
HTB1	Histone H2B	Strong
CDC34	Ubiquitin-conjugating enzyme (E2) and catalytic subunit of SCF ubiquitin-protein ligase complex	Strong
CHD1	Chromatin remodeller	Strong
CRM1	Major karyopherin, involved in export of proteins, RNAs, and ribosomal subunits from the nucleus	Strong
MOB1	Component of the mitotic exit network	Strong
DDC1	Member of the 9-1-1 clamp essential for the DNA damage response	Strong
CST6	Basic leucine zipper (bZIP) transcription factor	Strong
MPH1	Helicase that functions in an error-free DNA damage bypass pathway	Strong
YKL044w	Gene of unknown function	Strong
SIS2	Negative regulatory subunit of the protein phosphatase 1 Ppz1	Strong
UBC11	Ubiquitin-conjugating enzyme	Strong
NHP6A	High-mobility group non-histone chromatin protein	Strong
TPT1	tRNA 2'-phosphotransferase, catalyzes the final step in yeast tRNA splicing	Strong
TAF3	TFIID subunit involved in transcription initiation	Strong
RTT103	Involved in transcription termination	Medium
SRL3	Cytoplasmic protein that, when overexpressed, suppresses the lethality of a rad53 null mutation	Medium
RTT109	Histone H3 acetylase	Medium
GID8	Member of a ubiquitin ligase that carries out poly-ubiquitination.	Medium
GIS2	Protein with seven cysteine-rich CCHC zinc-finger motifs	Medium
RTT106	Histone chaperone	Medium
YOL114c	Gene of unknown function	Medium
IRC13	Null mutant displays increased levels of spontaneous Rad52 foci	Medium
NUP1	Nuclear pore complex (NPC) subunit, involved in protein import/export and in export of RNAs	Weak
PPS1	Protein phosphatase with specificity for serine, threonine, and tyrosine	Weak
UBP14	Ubiquitin-specific protease	Weak
NSE3	Essential subunit of the Mms21-Smc5-Smc6 complex	Weak
PAB1	Poly(A) binding protein, part of the 3'-end RNA-processing complex	Weak
DAM1	Essential subunit of the Dam1 complex, involved in kinetochore movement	Weak
CPD1	Cyclic nucleotide phosphodiesterase	Weak
UBP7	Ubiquitin-specific protease that cleaves ubiquitin-protein fusions	Weak
RPB4	Subunit of RNA polymerase involved in 3'-end RNA processing and export	Weak
RFX1	Major transcriptional repressor of DNA-damage-regulated genes	Weak
HAS1	ATP-dependent RNA helicase, localizes to nuclear pore	Weak
CNM67	Component of the spindle pole body outer plaque	Weak

by *Rtt109* and its deposition by *Rtt106* stabilize the advancing replication forks and allow the repair of lesions that occur during replication (Clemente-Ruiz *et al.* 2011).

CHD1: *Chd1* is a chromatin remodeler that belongs to the chromodomain-containing subfamily of *Snf2*-like proteins; these proteins are believed to be recruited through methylated histone lysine residues and to promote nucleosome remodeling. Interestingly, it was recently shown that *Chd1* is required for histone H2B ubiquitination, which is required for histone restoration after RNA polymerase passage (Lee *et al.* 2012).

NHP6A: *Nhp6A* is an abundant small protein that binds DNA nonspecifically and bends it sharply. By binding the minor groove of DNA with a single HMGB domain and wrapping around the DNA to contact the major groove, it helps remodeling the nucleosomes (Stillman 2010). A less-abundant, very similar protein, *Nhp6B*, exists. Both were found to be components, together with *Spt16p* and *Pob3p*,

of the FACT complex, which plays important roles in transcription and DNA replication (Ruone *et al.* 2003).

Thus, overexpression of histone H2B, or of proteins that promote nucleosome re-establishment after DNA or RNA polymerase transit, alleviate the SL phenotype of *elg1Δ* and *srs2Δ*. We can think of three possible models for this: (1) mutations in these genes cause replication fork instability, which can be suppressed by accelerating replication in the presence of excess histone dimers ready to be assembled into the newly replicated DNA. (2) Alternatively, *Srs2* and *Elg1* may play a direct role in the regulation of histone levels in the cell, and their SL is alleviated when histone levels are increased by other means. (3) A third model, involving an effect of these chromatin regulators in transcription regulation (which is also affected by histone levels and deposition), seems to us less likely, although recent evidence supports a role for histone levels in the regulation of promoter fidelity (Silva *et al.* 2012).

■ Table 3 GO annotation enrichment

Description	GOID	Ontology	Intersection	Category Size	P Value
Chromosome	GO:0005694	Cellular compartment	11	393	2.33E-06
Chromosomal part	GO:0044427	Cellular compartment	9	352	4.78E-05
Structure-specific DNA binding	GO:0043566	Molecular function	5	101	1.47E-04
Chromatin binding	GO:0003682	Molecular function	4	84	8.27E-04
Regulation of mitotic cell cycle	GO:0007346	Biological process	6	105	1.33E-05
Negative regulation of cellular process	GO:0048523	Biological process	10	422	3.17E-05
Negative regulation of biological process	GO:0048519	Biological process	10	432	3.86E-05
DNA repair	GO:0006281	Biological process	8	263	4.05E-05
DNA packaging	GO:0006323	Biological process	4	61	2.49E-04
Chromatin assembly or disassembly	GO:0006333	Biological process	4	66	3.36E-04
Nucleosome organization	GO:0034728	Biological process	4	69	3.97E-04
DNA conformation change	GO:0071103	Biological process	4	80	6.91E-04
Negative regulation of transposition	GO:0010526	Biological process	2	8	7.22E-04
Response to DNA damage stimulus	GO:0006974	Biological process	7	307	7.27E-04
Negative regulation of cellular metabolic process	GO:0031324	Biological process	7	307	7.27E-04
DNA replication-independent nucleosome organization	GO:0034724	Biological process	2	9	9.23E-04

An FDR correction of 0.001 was used

It is interesting to note that *elg1* mutants are synthetic sick with mutations in *HTB1* (data not shown), *RTT106* (Imbeault *et al.* 2008), *RTT109* (Bellaoui *et al.* 2003), although not with *chd1Δ*, *nhp6aΔ* or the double *nhp6aΔ nhp6bΔ* (data not shown). In contrast, *srs2Δ* shows a synthetic fitness phenotype when combined with *chd1Δ* (Pan *et al.* 2006), *rtt109Δ* (Costanzo *et al.* 2010), but not with *htb1Δ*, *rtt106Δ*, *nhp6aΔ* or the double *nhp6aΔ nhp6bΔ* (data not shown). Thus, the spectrum of synthetic phenotypes between *elg1Δ* and *srs2Δ* only partially overlap, consistent with nonoverlapping functions for the two genes.

Genes affecting RNA end-processing and/or nuclear transport: This category includes the following genes:

RTT103: Transcription by RNA polymerase II (PolII) and transcript processing are coordinated by the phosphorylation status of the PolII C-terminal domain (CTD). Transcription termination requires, surprisingly, the activity of RNA nucleases such as *Rat1*, which is recruited by *Rtt103* (Kim *et al.* 2004). This requires an interaction between *Rtt103* and the Ser2-phosphorylated form of PolII CTD. It was recently found that phosphorylation of the Tyr-1 residue in the CTD prevents *Rtt103* binding, thus enhancing transcription processivity. At the 3' end of the transcript Tyr-1 levels drop, allowing *Rtt103* binding and transcription termination followed by poly-adenylation (Mayer *et al.* 2012).

PAB1: *Pab1* associates with RNA poly(A) tails, mediating the interactions between the 5' cap structure and the 3' mRNA poly(A) tail. It plays a role in mRNA stability and stimulates the initiation of translation (Amrani *et al.* 2008; Amrani *et al.* 1997). In addition, *Pab1* is essential in the coupling of 3' RNA processing and nuclear export of the mRNA (Dunn *et al.* 2005).

RPB4: *Rpb4* is an RNA polymerase II subunit that forms a complex with *Rpb7*. Contrary to other subunits of RNA polymerase II, *Rpb4* is not essential under normal conditions; however, it becomes essential under stress. Lately it was found that this complex plays an important role in coordinating transcription in the nucleus with mRNA degradation and translation in the cytoplasm (Harel-Sharvit *et al.* 2010).

GIS2: *Gis2* binds to a subset of mRNA molecules and promotes their translation by a still poorly understood mechanism (Sammons *et al.* 2011; Tsvetanova *et al.* 2010). Both *Gis2* and its human ortholog, CNBP, mutations that cause muscular dystrophy, are part of stress granules (Rojas *et al.* 2012).

HAS1: The *HAS1* gene encodes an ATP-dependent RNA helicase that plays an important role in ribosomal RNA processing and nuclear export. Accordingly, it is highly enriched in nuclear pore complex fractions (Rout *et al.* 2000).

NUP1: The *Nup1* protein is a component of the central core of the nuclear pore complex. It plays a role in RNA and protein transport through the pore, by serving as a release factor for karyopherin (Aitchison and Rout 2012; Izaurralde and Adam 1998).

CRM1: *Crm1* is the main karyopherin protein, involved in export of proteins, RNAs, and ribosomal subunits from the nucleus. It plays a central role in cargo export (exportin function) (Aitchison and Rout 2012).

The common mechanism suggested by these genes is that the SL phenotype observed can be suppressed by increasing the levels of certain mRNA(s) as well as their transport from the nucleus (through the nuclear pore), perhaps causing an increase in the translation level of (a) target gene(s).

Spindle pole body and spindle checkpoints: **CNM67:** *Cnm67* is a component of the spindle pole body outer plaque. It plays a central role in spindle orientation and in mitotic nuclear migration (Brachat *et al.* 1998). *Cnm67* interacts physically and genetically with *Gis2* (Scherrer *et al.* 2011; Wilmes *et al.* 2008). It also interacts with several kinetochore components, and mutations in *CNM67* exhibit a synthetic fitness defect when combined with *srs2* mutations (Pan *et al.* 2006).

DAM1: *Dam1* is a member of the DASH complex, which facilitates microtubule-kinetochore interactions, thereby playing an essential role in chromosome segregation. The DASH complex forms a ring around microtubules that stabilizes the microtubule and promotes its growth (Miranda *et al.* 2005). It also allows the kinetochore to preferentially bind to the microtubule plus end (Miranda *et al.* 2005). *Dam1* is phosphorylated by the *Ipl1* Aurora kinase; although this phosphorylation does not seem to affect the binding activities of the complex, it is essential for the establishment of bipolar attachments. Notably, *Dam1* is also methylated by the *Set1* methyltransferase, the same enzyme in charge of methylating histone H3 at lysine4 (H3K4). Remarkably, both H3K4 and *Dam1* methylation depend on prior ubiquitination of histone H2B (Latham *et al.* 2011). Mutations in *DAM1* show synthetic fitness defects with mutations in *RTT103*.

MOB1: *Mob1* is a member of the mitotic exit network. It is located at the spindle pole body, where it binds and regulates the *Dbf2* protein

kinase. Activation by Cdc15p-dependent phosphorylation leads to a change in its location, inducing cytokinesis and cell separation (Mah *et al.* 2001). Interestingly, Mob1 is important for the proper localization of the Ipl1 Aurora kinase (Stoepe *et al.* 2005), which phosphorylates Dam1. Thus, mitotic exit network activity is important for proper kinetochore activity, and controls the spindle checkpoint (Tan *et al.* 2005).

The mechanism by which these genes may suppress the SL of *elg1Δ srs2Δ* cells could be related to the spindle checkpoint, which gets activated as a secondary response to DNA damage and prevents adaptation to DNA damage in *srs2Δ* mutants (Dotiwala *et al.* 2010).

Protein modification: A number of genes encoding protein modifiers were identified; these include ubiquitin-related proteins, as well as a protein phosphatase and a regulator of another phosphatase. The targets of these proteins that allow suppression of the SL phenotype is still unclear.

CDC34: Cdc34 encodes a ubiquitin-conjugating enzyme (E2) that is the catalytic subunit of the SCF ubiquitin-protein ligase complex. This complex is in charge of targeting proteins for degradation, particularly those involved in cell cycle progression. Cdc34 levels are increased under conditions of DNA replication stress, suggesting that it plays an important role in coping with this type of genotoxic situation (Tkach *et al.* 2012).

UBC11: Ubc11 is a ubiquitin-conjugating enzyme (E2). Its E3 partners, targets or functions are currently unknown.

GID8: Gid8 is part of another multiprotein complex that functions as an E3 ubiquitin ligase to ubiquitinate proteins to send them to degradation by the proteasome (Menssen *et al.* 2012).

UBP14: Ubp14 is a deubiquitinating protein that disassembles free poly-ubiquitin chains, thus controlling the rate of degradation by the proteasome (Amerik *et al.* 1997). Interestingly, Ubp14 collaborates with the Gid complex (Regelmann *et al.* 2003) and mutations in this gene are synthetic lethal with mutations in Cdc34 (Cocklin *et al.* 2011).

UBP7: Ubp7 is another deubiquitination enzyme of unknown function.

PPS1: Pps1 is a phosphatase that plays a role during S-phase (Ernsting and Dixon 1997). Its targets remain unknown.

SIS2: Sis2/Hal3 binds to the C-terminal catalytic domain of the serine/threonine phosphatase Ppz1 and strongly inhibits its activity (de Nadal *et al.* 1998). Decreased activity of Ppz1 leads to salt tolerance, compromised cell integrity and accelerated G1/S transition (Munoz *et al.* 2003; Posas *et al.* 1995). In addition, SIS2/HAL3 has a role in the biosynthetic pathway of coenzyme A, as part of a different complex, the PPCDC (phosphopantothienoylcysteine decarboxylase complex), which also includes the Cab3 and Vhs3 subunits (Ruiz *et al.* 2009). However, transformation of the *elg1Δ srs2Δ* strain with plasmids overexpressing these two subunits conferred no suppression. We thus conclude that it is likely that its interaction with Ppz1 phosphatase is the one responsible for the suppression (although we cannot rule out the possibility that Sis2, and only Sis2, is the limiting factor in the activity of the CoA synthesis complex). Overexpression of a phosphatase repressor may increase the level of a phosphorylated protein, which may be limiting in the *elg1Δ srs2Δ* strains.

tRNA splicing: Two genes were found, encoding successive steps in tRNA splicing.

TPT1: TPT1 is an essential gene that encodes tRNA 2'-phosphotransferase, which catalyzes the final step of tRNA splicing

(Culver *et al.* 1997). tRNA splicing is carried out by a multisubunit endonuclease, followed by ligation. Tpt1 catalyzes the transfer of the splice junction 2'-phosphate from the ligated tRNA to NAD to produce ADP-ribose 1''-2'' cyclic phosphate (Appr > p) (Culver *et al.* 1997). In a conditional lethal *tpt1* mutant, 2'-phosphorylated tRNAs accumulate, and the tRNAs show altered posttranscriptional modification (Spinelli *et al.* 1997).

CPD1: Each tRNA splicing reaction produces an equimolar amount of ADP-ribose 1'',2''-cyclic phosphate (Appr > p). This molecule is converted into ADP-ribose-1''-phosphate (Appr1p) by Cpd1 (Shull *et al.* 2005). It is estimated that ~500 000 tRNA splicing events take place per *Saccharomyces cerevisiae* generation (Shull *et al.* 2005).

The significance of having found these two related enzymes in our screen is not clear. One possibility is that Appr1p or its derivatives may serve as signaling molecules in the cell, and an increase in its level may somehow be beneficial. We note that Tpt1 was identified as a histone H3-interacting protein (Gilmire *et al.* 2012).

Transcription factors: The genes in this category are as follows:

CST6: CST6 was identified in a search for genes that are important for chromosome stability (CST genes) (Ouspenski *et al.* 1999). Interestingly, this screen also identified, among others, PAB1, as well as ubiquitin and histone genes (Ouspenski *et al.* 1999). Cst6 is a transcription factor activator, a member of the *S. cerevisiae* ATF/CREB family (Garcia-Gimeno and Struhl 2000). Its targets are still unknown.

RFX1: RFX1 encodes the major transcriptional repressor of some DNA-damage-regulated genes. It helps recruit the repressors Tup1 and Cyc8 to the promoters of the relevant genes, such as the subunits of the ribonucleotide reductase enzyme. Upon DNA damage Rfx1 becomes phosphorylated and is released from the genes, allowing the recruitment of SAGA or TFIID subunits required for their expression (Ghosh and Pugh 2011).

TAF3: Taf3 is a subunit of the TFIID complex, required for gene expression. It has lately been found that TFIID is preferentially recruited to promoters that lack TATA boxes, whereas SAGA binds preferentially TATA-containing regulatory sequences (Rhee and Pugh 2012).

The detection of these genes, involved in transcriptional regulation, as suppressors of the SL phenotype of *elg1Δ* and *srs2Δ*, implies that the suppression is possible by transcriptional activation or repression of target genes. However, the identity of the regulated genes, albeit, is still to be found.

Additional genes: BAP2: Bap2 encodes a high-affinity leucine permease that can also function as a branched-chain amino acid permease involved in the uptake of isoleucine and valine (Grauslund *et al.* 1995).

SRL3: The SRL3 gene was identified in a screen for high copy number suppressors of the essential function of the DNA damage response genes MEC1 (ATR in humans) and RAD53 (CHK2) (Desany *et al.* 1998). Its exact function remains enigmatic.

IRC9, IRC13: These two genes were identified in a screen for mutants exhibiting increased levels of Rad52 foci. Both are considered "dubious ORFs" by the Saccharomyces Gene Database (<http://www.yeastgenome.org>).

YOL114c: YOL114c is an uncharacterized ORF.

YKL044w: This small ORF is present within the UTR of the PHD1 gene. Evidence for its being translated comes from ribosomal profiling experiments (Brar *et al.* 2012).

In summary, we have carried out a screen for genes that, when overexpressed, can suppress the SL of an *elg1Δ srs2Δ* strain. We did not isolate plasmids carrying the *ELG1* or *SRS2* genes; however, this is an expected result: overexpression of these genes is slightly toxic to the cells, and these genes tend to be underrepresented when screening high copy number libraries (Leon Ortiz *et al.* 2011; Parnas *et al.* 2009). The coverage used (>x20) allowed us to identify a relatively large number of candidate genes, which were confirmed one by one by re-cloning and re-testing. Although most genes were identified only once, several of the genomic regions were independently isolated several times, and some (e.g., *PAB1*) were identified six times. Thus, we are confident that our results represent a significant fraction of the genes that affect the genetic interactions between *SRS2* and *ELG1*. It appears that there is a large number of ways in which the SL phenotype can be bypassed. Our results suggest that chromatin modification and sister chromatid cohesion are processes related to the function defective in *elg1Δ srs2Δ* double mutants. In addition, increased transcription/translation and nuclear export seem to be able to bypass the synthetic phenotype, possibly by affecting an effector protein(s). We believe that the results obtained from this overexpression screen will provide valuable information for further research needed to better understand the functions carried out by these genes and their interactions.

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