Yeast hEST1A/B (SMG5/6)–Like Proteins Contribute to Environment-Sensing Adaptive Gene Expression Responses

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ABSTRACT During its natural life cycle, budding yeast (Saccharomyces cerevisiae) has to adapt to drastically changing environments, but how environmental-sensing pathways are linked to adaptive gene expression changes remains incompletely understood. Here, we describe two closely related yeast hEST1A-B (SMG5-6)–like proteins termed Esl1 and Esl2 that contain a 14-3-3–like domain and a putative PilT N-terminus ribonuclease domain. We found that, unlike their metazoan orthologs, Esl1 and Esl2 were not involved in nonsense-mediated mRNA decay or telomere maintenance pathways. However, in genome-wide expression array analyses, absence of Esl1 and Esl2 led to more than two-fold deregulation of transcripts, most of which were expressed inversely to the appropriate metabolic response to environmental nutrient supply; for instance, normally glucose-repressed genes were derepressed in *esl1Δ* esl2Δ double mutants during growth in a high-glucose environment. Likewise, in a genome-wide synthetic gene array screen, *esl1Δ* esl2Δ double mutants were synthetic sick with null mutations for Rim8 and Dfg16, which form the environmental-sensing complex of the Rim101 pH response gene expression pathway. Overall, these results suggest that Esl1 and Esl2 contribute to the regulation of adaptive gene expression responses of environmental sensing pathways.

hEST1A/B (SMG5/6) are structurally closely related bifunctional metazoan proteins with roles in telomere maintenance and in the nonsense-mediated mRNA decay (NMD) pathway that degrades mRNAs containing premature stop codons during quality-control pioneer rounds of translation in the nucleus. hEST1A/B (SMG5/6) contain a central 14-3-3–like domain (Fukuhara et al. 2005) that may mediate protein–protein interactions for regulation of the key NMD factor UPF1 (Anders et al. 2003; Chiu et al. 2003; Ohnishi et al. 2003) and a C-terminal PilT N-terminus (PIN) domain that provides endoribonuclease activity toward degradation of NMD substrates (Eberle et al. 2009; Huntzinger et al. 2008). Another related protein, hEST1C (SMG7), also contains a central 14-3-3 domain but lacks the C-terminal PIN domain. NMD proteins such as hEST1A/B (SMG5/6) are highly enriched at telomeres (Reichenbach et al. 2003; Snow et al. 2003) and negatively regulate the expression of telomeric repeat–containing RNA (Schoeftner and Blasco 2008), which may explain the crosstalk between NMD and telomere maintenance pathways.

The name hEST1A-C relates to the similarity of these proteins to the yeast telomerase subunit Est1 within the 14-3-3–like domain, which therefore is also referred to as the Est-one-homology domain (Beernink et al. 2003; Chiu et al. 2003; Reichenbach et al. 2003; Snow et al. 2003). However, it recently has been shown that the yeast NMD...
factor Ebs1 is the structural and functional ortholog of hEST1C (SMG7) (Luke et al. 2007), and no yeast counterparts for hEST1A/B (SMG5/6) previously have been identified. During database mining attempts to identify potential cofactors of a yeast protein with DNA damage and telomere-related functions, Mdt1/Pim1 (Pike and Heierhorst 2007; Pike et al. 2004; Traven et al. 2010), we noticed that a large-scale two-hybrid screen (Uetz et al. 2000) had found it to interact with two uncharacterized Est-one-homology and PIN domain–containing open reading frames Yil151c and Ykr096w. Here, we show that Yil151c and Ykr096w are structural orthologs of hEST1A–B/SMG5–6 and have thus named them Esl1 and Esl2 (EST = EST/SMG–like). Surprisingly, we found that Esl1 and Esl2 have no apparent telomere-related or NMD functions but instead are involved in the expression of a small subset of genes, including hexose and amino acid metabolism–related genes, during adaptation to nutrient supply by the environment.

**MATERIALS AND METHODS**

**Yeast strains**

All yeast strains used in this study are listed in Table 1 and were derived from W303-1A, unless otherwise indicated. Gene disruptions and C-terminal tagging were performed using a technique mediated by polymerase chain reaction (PCR) (Longtine et al. 1998). Nuclease-dead mutants were generated by PCR-based site-directed mutagenesis derived from W303-1A, unless otherwise indicated. Gene disruptions were performed in YPD medium (1% yeast extract, 2% peptone, 2% glucose) at 30°C, except for selection against petites, for which cells were plated on YPG (1% yeast extract, 2% peptone, 3% glycerol).

**Solid medium plate assays**

Overnight cultures were diluted to a starting density of A600 = 0.5 and were spotted in 10-fold serial dilutions onto YPD plates or medium containing various concentrations of drugs as indicated. Plates were incubated for 3–5 days at 30°C.

**Nucleic acids blots**

Cellular DNA and RNA were prepared by phenol–chloroform extraction. RNA was separated by electrophoresis at 80 V in 1.2% (w/v) agarose gels containing 1× MOPS buffer and 6.3% formaldehyde with buffer recirculation. Agarose gels for DNA analysis contained 0.5× TAE. Nucleic acids were transferred overnight by capillary transfer to nylon membranes using 10× SSC buffer. Membranes were incubated with radioactively labeled probes, exposed to phosphorimager screens, and analyzed using Molecular Dynamics ImageQuant software. For analysis of telomere lengths, genomic DNA was subjected to XhoI restriction endonuclease digestion at 37°C for 4 hr as described (Pike and Heierhorst 2007; Traven et al. 2010).

**Synthetic genetic array analysis**

The screen for synthetic sick/lethal interactions was performed for the query strain *esl1Δ esl2Δ* (Y1099) according to the method described (Tong and Boone 2006). Positive interactions from the screen were individually validated by tetrad dissections on YPD plates.

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**Table 1 Yeast strains used in this study**

| Strain     | Genotype          | Reference       |
|------------|-------------------|-----------------|
| Y52 (W303-1a) | MATa ade1-1 can1-100 leu2-3, 122 trp1-1 ura3-1 RAD5 | Zhao et al. 1998 |
| Y829       | Y52 esl1Δ::KAN    | This study      |
| Y830       | Y52 esl2Δ::NAT    | This study      |
| Y831       | Y52 esl1Δ::KAN esl2Δ::NAT | This study |
| Y1113      | Y52 upf1Δ::URA3   | This study      |
| Y1115      | Y52 esl1Δ::KAN upf1Δ::URA3 | This study |
| Y1117      | Y52 esl2Δ::NAT upf1Δ::URA3 | This study |
| Y1119      | Y52 esl1Δ::KAN esl2Δ::NAT upf1Δ::URA3 | This study |
| Y1280      | Y52 esl1-nd       | This study      |
| Y1282      | Y52 esl2-nd       | This study      |
| Y1284      | Y52 esl2-nd       | This study      |
| Y1333      | Y52 trf4Δ::NAT    | This study      |
| Y1335      | Y52 rimΔ::NAT     | This study      |
| Y1342      | Y52 esl1-nd esl2-nd | This study |
| Y1344      | Y52 esl1-nd esl2-nd | This study |
| Y996 (Y7092)| MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 lyp1Δ cyh2 can1Δ::STE2pr-SpHIS5 | Tong and Boone 2006 |
| Y1099      | Y996 esl1Δ::NAT esl2Δ::URA3 | This study |
| Y32 (BY4741)| MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 | Brachmann et al. 1998 |
| Y1289      | Y32 esl1Δ::NAT    | This study      |
| Y1290      | Y32 esl2Δ::URA3   | This study      |
| Y1291      | Y32 esl1Δ::NAT esl2Δ::URA3 | This study |
| Y1407      | Y32 esl1-nd       | This study      |
| Y1408      | Y32 esl1-nd       | This study      |
| Y1410      | Y32 esl1-nd       | This study      |
| Y1417      | Y32 esl1-nd esl2-nd | This study |
| Y1418      | Y32 esl1-nd esl2-nd | This study |
| Y219 (JKM179)| ade1 leu2-3,112 lys5 trp1::hisG ura3-52 hmlΔ::ADE1 hmrΔ::ADE1 ade3:: GAL-HO | Lee et al. 1998 |
| Y496 (TGI354) | ade1 leu2-3,112 lys5 trp1::hisG ura3-52 hmlΔ::ADE1 hmrΔ::ADE1 ade3:: GAL-HO MATa-inc arg5,6::MATa-HPH | Ira et al. 2003 |
Senescence assays
Sporulation cultures were digested with Zymolase 20T in sorbitol buffer and tetrads were dissected on YPD plates using a dissection microscope; 10^5 cells of freshly dissected spores were allowed to grow for 24 hr in YPD media at 30°. In exactly 24-hr intervals, cell densities were determined by hemocytometer counts of sonicated aliquots before redilution to 10^5 cells/ml. Approximately 200–400 cells were plated on YPD each day and colonies were counted after 3 to 4 days.

Multiple sequence alignment
The Basic Local Alignment Search Tool on the National Center for Biotechnology Information web site (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to identify regions of similarity between biological sequences. Multiple sequence alignments were generated with ClustalW on the European Bioinformatics Institute web site (http://www.ebi.ac.uk/Tools/msa/clustalw2/). All conserved and similar residues in the multiple sequence alignments were shaded using BoxShade 3.2 on the Swiss EMNet server (http://www.ch.embnet.org/software/BOX_form.html).

DNA microarray
Total RNA was prepared from YPD log-phase cultures of wild-type and esl1Δ esl2Δ double mutants in the W303-1A background. cDNA synthesis and two-color hybridization on yeast 8×15K format slides were performed by the Ramaciotti Centre for Gene Function Analysis (University of New South Wales). Data analysis was performed using the GeneSpring software (Agilent). Gene ontology enrichment analysis was performed using FuncAssociate 2.0 software (Berritz et al. 2009). The array data have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO accession number GSE48956).

Reverse-transcription PCR
Reverse-transcription PCR was performed using the method described previously (Beilharz and Preiss 2009).

RESULTS
Identification of Esl1 and Esl2 as yeast orthologs of hEST1A-B/SMG5-6
During database searches we noted that two of the reported Mdt1-interacting proteins (Uetz et al. 2000), the previously uncharacterized yeast open reading frames Yil151c and Ykr096w, share >70% similarity with each other along their entire polypeptide sequence. Interestingly, during Basic Local Alignment Search Tool searches for metazoan orthologs, we noticed that these two proteins also share extensive similarity (~45% overall) with human hEST1A/B and Dro sophila and Caenorhabditis elegans SMG5/6 proteins. Importantly, this similarity encompassed the region corresponding to the 14-3-3-like Est-one-homology domain (45–51% similarity; Figure 1, A and B) and the C-terminal PIN endonuclease domain (49–53% similarity; Figure 1, A and C) with complete conservation of four critical D/E residues required for nuclease activity of the PIN-domain proteins (asterisks in Figure 1C). Based on the structural similarities to HEST1A-B/SMG5-6, we have named Yil151c and Ykr096w Esl1 and Esl2 (ESL = EST/SMG-like), respectively.

Esl1 and Esl2 do not have telomere-related or NMD-related functions
To test if the structural similarities extend to similar protein functions, we monitored esl1Δ and esl2Δ single-null and esl1Δ esl2Δ double-null mutants for telomere-related and NMD-related defects (Pike and Heierhorst 2007; Traven et al. 2010). Telomere length in several independent esl1Δ, esl2Δ and esl1Δ esl2Δ clones were within the range of the wild-type, in contrast to rad50Δ mutants, which were included as a control for very short but stable telomeres (Figure 2, A and B), indicating that ESL1 and ESL2 do not contribute to normal telomere-dependent telomere length control. In the absence of telomerase, cells progressively senesce until a small subpopulation of so-called postsenescence survivors emerges that has switched to recombination-dependent alternative lengthening of telomeres pathways (Lundblad and Blackburn 1993). To determine if Esl1 and Esl2 and/or mutants for telomere-related and NMD-related defects (Pike and Heierhorst 2007; Traven et al. 2010). Telomere length in several independent esl1Δ, esl2Δ and esl1Δ esl2Δ clones were within the range of the wild-type, in contrast to rad50Δ mutants, which were included as a control for very short but stable telomeres (Figure 2, A and B), indicating that ESL1 and ESL2 do not contribute to normal telomere-dependent telomere length control. In the absence of telomerase, cells progressively senesce until a small subpopulation of so-called postsenescence survivors emerges that has switched to recombination-dependent alternative lengthening of telomeres pathways (Lundblad and Blackburn 1993). To determine if Esl1 and Esl2 are involved in alternative lengthening of telomeres, we deleted the gene for the catalytic subunit of telomerase, EST2, for senescence assays. However, the kinetics of the onset of senescence and the emergence of postsenescence survivors with normal proliferative capacity and colony formation were similar for esl1Δ esl2Δ est2Δ triple mutants compared with est2Δ alone (Figure 2C), and both cases of postsenescent colonies predominantly comprised the more efficient type II survivors subtype (data not shown). Taken together, these results indicate that Esl1 and Esl2 are not required for telomerase-dependent or alternative telomere maintenance mechanisms.

Apart from telomere length regulation, telomere-associated proteins may be involved in maintaining the heterochromatin structure of telomeres and transcriptional repression of telomerase-proximal genes (Baur et al. 2001; Blasco 2007; Gottschling et al. 1990). To assess if Esl1 and Esl2 affect telomere structure, we monitored 5′-fluororotic acid (5-FOA) sensitivity of strains containing a subtelomeric URA3 reporter gene. Ura3 converts 5-FOA to a toxic metabolite and, consequently, yku70Δ control cells that are unable to silence the subtelomeric URA3 reporter (Gottschling et al. 1990) were unable to grow on 5-FOA plates (Figure 2D). In contrast, esl1Δ esl2Δ mutants were able to grow on 5-FOA similar to the wild-type, and deletion of ESL1 and ESL2 did not affect the 5-FOA sensitivity (Figure 2D). In addition, there was no accumulation of natural subtelomere or telomere-derived transcripts, such as Y′-help and telomeric repeat-containing RNAs, in esl1Δ, esl2Δ and esl1Δ esl2Δ mutants (Figure 2E). Thus, Esl1 and Esl2 appear to be dispensable for maintenance of telomere structure.

To determine if Esl1 and Esl2 have NMD functions, we first measured expression levels of the endogenous nonsense-mutated ade2-1 locus. In contrast to the bona fide NMD-deficient upf1Δ control (He et al. 1997), there was no accumulation of ade2-1 transcripts in esl1Δ and/or esl2Δ mutants (Figure 2F). The NMD pathway also degrades unspliced transcripts and, similar to the ade2-1 mutant, there was no accumulation of the unspliced pre-CYH2 mRNA in esl1Δ and/or esl2Δ mutants (Figure 2G). Thus, based on these two independent assays, ESL1 and ESL2 do not seem to have NMD-related functions.

ESL1 and ESL2 contribute to some genome stability functions in a nuclease domain–dependent manner
Cells containing the ade2-1 nonsense mutation have a pink color (Figure 3A, WT). During routine propagation of esl1Δ esl2Δ double mutants, we noticed that culture plates had an increased incidence of white colonies, which is often attributable to the spontaneous accumulation of mitochondrial DNA mutations (Zhao et al. 1998) (Figure 3, A and B). All white esl1Δ esl2Δ colonies failed to grow on YP-glycerol plates (Figure 3C) on which respiratory-deficient mitochondrial petite mutants are unviable, indicating that ESL1 and ESL2 contribute to maintenance of mitochondrial genome stability.

To test if Esl1 and Esl2 may be involved in additional genome stability functions, we monitored their sensitivity to a range of genotoxic agents. In drop tests on plates containing DNA-damaging agents,
and/or \( \text{esl2} \Delta \) mutants grew \(-100\)-fold better in the presence of 0.2 \( \mu \text{g/ml} \) bleomycin, \(-10\)-fold better on 250 \( \mu \text{M} \) hydroxyurea, and \(-10\)-fold worse on 150 \( \mu \text{g/ml} \) adriamycin compared with the wild-type (Figure 3D). In all cases, the effect was more pronounced when \( \text{esl1} \) and \( \text{esl2} \) were simultaneously deleted, suggesting functional redundancy between the two proteins. To determine if these phenotypes were attributable to a potential PIN nuclease function, similar drop tests were performed using “nuclease-dead” \( \text{esl1} \) and \( \text{esl2} \) mutants containing amino acid substitutions of at least one of the four critical conserved residues whose mutation previously has been shown to abrogate the nuclease activity of other PIN domain proteins (Dziembowski et al. 2007; Schneider et al. 2007; Skruzny et al. 2009). In these assays, the nuclease-dead mutants phenocopied the bleomycin sensitivity of the \( \text{esl1} \Delta \) or \( \text{esl2} \Delta \) mutants (Figure 3E). Altogether, the results indicate that Esl1 and Esl2 contribute to the maintenance of

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**Figure 1** Comparison of Esl1 and Esl2 with metazoa hEST1A/B (SMG6/5) proteins. (A) Schematic illustration of domain topology of Esl1, Esl2, Est1, and hEST1A-C. (B) Multiple sequence alignment of the Est-one-homology (EOH) domains of \( S. \text{cerevisiae} \) Esl1, Esl2, Est1, hEST1A, \( D. \text{melanogaster} \) SMG6, and \( \text{C. elegans} \) SMG6. (C) Multiple sequence alignment of the PIN nuclease domains of \( S. \text{cerevisiae} \) Esl1, Esl2, Est1, hEST1A, \( D. \text{melanogaster} \) SMG6, and \( \text{C. elegans} \) SMG6. The four conserved acidic residues required for nuclease function. For a similar alignment of Esl1 and Esl2 with the other budding yeast PilT N-terminus (PIN) domain–containing proteins, see Rüther et al. 2006.
genomic redundancy between Esl1 and Esl2. Interestingly, two mutants compared with single mutants (Figure 4), indicative of three independent experiments. Analysis in (F) normalized to actin levels. Results are means ± SE from three independent experiments.

Figure 2 Esl1 and Esl2 do not have telomere-related or nonsense-mediated mRNA decay (NMD)-related functions. (A) Schematic illustration of assays to measure telomere length and gene expression. Xhol restriction sites and Y'-Xhol-ter probe used for Southern analysis are indicated. The probe detects the 5.2-kb Y'-short element, 6.7-kb Y'-long element, and the terminal restriction fragment (TRF). The Y'-help1 probe used for northern analysis in (E) is also indicated. (B) Southern blot analysis of independent clones of wild-type (WT), est1Δ, est2Δ, and est2ΔΔ. (C) Cultures were inoculated with ~10^6 cells/ml and back-diluted to 10^5 cells/ml in exactly 24-hr intervals (right). Approximately 200–400 cells from daily cultures in the left panel were plated on YPD. Plates were incubated for 3 days at 30°C and percentage of plated cells able to form a colony was determined (left). Results are means ± SE from three independent wild-type (WT), est1Δ, est1ΔΔ, and est2ΔΔ clones and seven independent est2Δ and est1Δ est2Δ clones. (D) Ten-fold serial dilutions of WT, yku70Δ, est1Δ est2Δ, and yku70Δ est1Δ est2Δ were spotted onto YPD and 5'-fluororotic acid (5'-FOA) plates. Plates were incubated 3–4 days at 30°C. (E) Northern blot analysis of telomeric repeat-containing RNAs (TERRAs; measured by the Y'-Xhol-ter probe) and Y' element encoded helicase (measured by the Y'-help1 probe). ACT1 is used as the loading control. Note that no TERRA signal was detectable in any of the strains under basal conditions. (F) Northern analysis to measure NMD substrate levels in WT, est1Δ, est1ΔΔ, est1Δ est2Δ, and upf1Δ. (G) Quantification of northern analysis in (F) normalized to actin levels. Results are means ± SE from three independent experiments.

Loss of ESL1 and of ESL2 lead to impaired genetic fitness with trf4Δ, rim8Δ, and dfg16Δ
As an unbiased approach to identify possible cellular functions of Esl1 and Esl2, a synthetic gene array screen was performed. For this purpose, an est1Δ est2Δ double-mutant strain was mated with the complete set of haploid-viable deletion yeast deletion mutants (Tong and Boone 2006), sporulated, and then plated on three different types of selective media to detect synthetic genetic interactions of est1Δ or est2Δ single mutants and est1Δ est2Δ double mutants. In the high-throughput screening format, est1ΔΔ was synthetic sick with four other deletions, est2ΔΔ was sick or lethal with 12 other deletions, and est1Δ est2Δ double mutants were synthetic sick or lethal with another seven gene deletions (Table 2). The genetic interactions identified by this approach are enriched in the functional categories phosphatidylinositol-3-phosphate binding (GO:0032266; adjusted p = 0.004) and endosome (GO:0005768; adjusted p = 0.008). Surprisingly, only three of the interactions, with trf4Δ, rim8Δ, and dfg16Δ, also were observed by manual tetrad dissection analysis on rich YPD medium (Figure 4), presumably because the less restrictive growth conditions compared with synthetic medium (plus antibiotics) select against somewhat weaker genetic interactions. Moreover, in all three cases, the synthetic growth defect on dissection plates was stronger with est1Δ est2Δ double mutants compared with single mutants (Figure 4), indicative of functional redundancy between Esl1 and Esl2. Interestingly, two of these ESL1 and ESL2 interactors, Dfg16 and Rim8 (Figure 4B), also physically interact as the G-protein-coupled receptor and β-arrestin-like adaptor in the Rim101 pathway that regulates the expression of pH-responsive genes in the adaptive response to alkaline environments (Lamb and Mitchell 2003; Lamb et al. 2001; Lin et al. 2008). However, Trf4 (Figure 4A) is a noncanonical poly(A) polymerase that forms part of the TRAMP complex involved in exosome-dependent
RNA degradation (LaCava et al. 2005; Vanacova et al. 2005; Wyers et al. 2005), which is interesting in view of the notion that all other PIN domain-containing proteins characterized to date exert ribonuclease activity in vitro and/or in vivo (Bleichert et al. 2004; Eberle et al. 2009; Fatica et al. 2004; Huntzinger et al. 2008; Schaeffer et al. 2009).

**Deregulation of hexose and one-carbon metabolism genes in esl1Δ esl2Δ mutants**

Based on the genetic interaction with trf4Δ and the presumed PIN-mediated RNase function of Esl1/2, we performed a genome-wide RNA microarray analysis of esl1Δ esl2Δ double mutants compared with the wild-type to identify potential Esl1 and Esl2 targets. In total, the expression levels of 53 genes were altered by at least two-fold in esl1Δ esl2Δ cells, with 30 genes that were upregulated and 23 genes that were downregulated (Figure 5). The most highly enriched gene ontology terms associated with the deregulated genes include glycine metabolic process and carbohydrate transport (GO:0006544 and GO:0008643, each with adjusted p < 0.001). The 10 most highly upregulated or downregulated genes are indicated in Figure 6A, and their genomic contexts are shown in Figure 6, B and C. Upregulated expression was confirmed by semiquantitative reverse-transcription PCR analyses for the transcripts of HXT6/HXT7 (which are too similar to be distinguishable by reverse-transcription PCR; Figure 6D), PHO89 (Figure 6E), and HXK1 (Figure 6F). In case of Hxk1, a similar upregulation of the protein was confirmed for two independent esl1Δ esl2Δ cultures compared with the wild-type by Western blot analysis (Figure 6G).

The most represented gene ontology terms among deregulated transcripts in esl1Δ esl2Δ mutants are in the classes of transport, carbohydrate metabolism, and one-carbon metabolism (Figure 5). Several hexose transporters involved in glucose uptake were deregulated in esl1Δ esl2Δ mutants. Strikingly, the high-affinity glucose transporters HXT6 and HXT7, which are normally induced under low glucose conditions, were upregulated during growth in high-glucose medium, whereas the low-affinity transporter HXT5, which is normally expressed under high glucose conditions, was downregulated (Figure 5). Likewise, the hexokinase Hxk1 (Figures 5 and 6, F and G) and several other genes that are usually repressed in high glucose were derepressed in our mutants, including the MAL genes (MAL12, MAL32, and MAL33) that are involved in maltose transport and metabolism, the lactate transporter Jen1, and genes involved in glycogen metabolism (GPH1 and PGM2). Similarly, even though the cells were grown in the presence of high levels of glycine, several transcripts of the one-carbon regulon—the glycine decarboxylase complex (GCV1, GCV2, and GCV3), the aminocarboxyamide ribotide transformylase

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**Table 2 Synthetic genetic interactions of esl1 and esl2 in a synthetic gene array screen**

| ORF     | Gene   | esl1Δ | esl2Δ | esl1Δ | esl2Δ |
|---------|--------|-------|-------|-------|-------|
| YB016W  | FUS3   | Sick  | ND    | ND    | ND    |
| YR023W  | DAL81  | Sick  | ND    | ND    | ND    |
| YL036W  | SNX4   | Sick  | ND    | ND    | ND    |
| YOL115W | TRF4   | Lethal| ND    | ND    | ND    |
| YB026C  | ETR1   | ND    | Sick  | Sick  | Sick  |
| YBR131W | CCZ1   | ND    | Sick  | Sick  | Sick  |
| YCR063W | BUD31  | ND    | Sick  | Sick  | Sick  |
| YDR074W | TPS2   | ND    | Sick  | Sick  | Sick  |
| YGL212W | VAM7   | ND    | Sick  | Sick  | Sick  |
| YJL204C | RCY1   | ND    | Sick  | Sick  | Sick  |
| YOR106W | VAM3   | ND    | Sick  | Sick  | Sick  |
| YOR030W | DFG16  | ND    | Sick  | Sick  | Sick  |
| YOR132W | VPS17  | ND    | Sick  | Sick  | Sick  |
| YOL012C | HTZ1   | ND    | Sick  | Sick  | Sick  |
| YDR080W | VPS41  | ND    | Sick  | Sick  | Sick  |
| YER071C | YER071C| ND    | Sick  | Sick  | Sick  |
| YFL010C | WWM1   | Normal| Sick  | Sick  | Sick  |
| YGL050W | TWW3   | Normal| Sick  | Sick  | Sick  |
| YGL045W | RIM8   | Normal| Sick  | Sick  | Sick  |
| YGR164W | YGR164W| Normal| Sick  | Sick  | Sick  |
| YFL003C | MSH4   | Normal| Lethal| Lethal| Lethal|
| YOL116W | MSN1   | Normal| Lethal| Lethal| Lethal|
| YDR293C | SSD1   | Normal| Lethal| Lethal| Lethal|

Sick/lethal interactions with single or double mutants were screened and scored visually. ND, not determined.

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Figure 3 Genome stability functions of Esl1 and Esl2. (A) Freshly sporulated cultures were plated on YPD and plates were incubated for 3–4 days at 30°C. (B) Quantification of the percentage of white colonies formed on YPD plates for the indicated genotypes. Data are means ± SE. *p < 0.005, n = 21, two-tailed Student t test. (C) Seven randomly picked white petite-like colonies (W) and a single red colony (R) were restreaked on glucose (left) and glycerol (right) plates. (D and E) Ten-fold serial dilutions of the indicated strains were spotted onto YPD plates and YPD plates containing the indicated concentrations of various DNA-damaging drugs. The esl1-nd mutant carries two amino acid substitutions (D952N and E982Q) while the esl2-nd mutant carries a single amino acid substitution (D1123N). Plates were incubated for 3–4 days at 30°C. WT, wild-type.

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With the wild-type to identify potential Esl1 and Esl2 targets. In total, RNA microarray analysis of...
levels were not altered in the nuclease-deficient alleles compared with the wild-type (Figure 6, D and F); in the case of PHO89, upregulation in esl1-nd esl2-nd mutants was attenuated compared with esl1Δ esl2Δ (Figure 6E). Thus, these data indicate that Esl1 and Esl2 may regulate the expression of the altered transcripts in a largely nuclease-independent manner.

**Nuclease-independent deregulation of noncoding transcripts in esl1Δ esl2Δ mutants**

During the analysis of the 10 most highly upregulated or downregulated transcripts in esl1Δ esl2Δ mutants (Figure 6A), we noticed a striking association of these genes with neighboring noncoding so-called cryptic unstable transcripts (CUTs) and stable unannotated transcripts (SUTs) (Figure 6, B and C). Because some of these noncoding RNAs are normally degraded in a TRAMP complex-dependent manner (Xu et al., 2009), we wondered whether deregulation of SUTs and CUTs might explain the synthetic sickness of esl1Δ esl2Δ with trf4Δ (Figure 4A). Interestingly, reverse-transcriptase PCR analysis confirmed upregulation of most of the CUTs and SUTs near HXT7/HXT6, PHO89, and HXK1 loci in esl1Δ esl2Δ cells (Figure 6, D–F). However, these changes in transcript levels were again independent of Esl1 and Esl2 nuclease domain integrity (Figure 6, D–F). For comparison, loss of TRF4 seemed to have an overall similar effect as esl1Δ esl2Δ on the expression of the adjacent CUTs and SUTs but did not affect the expression of the Esl1-regulated and Esl2-regulated coding genes (Figure 6, D and E). Thus, the data suggest that deregulation of adjacent CUTs and SUTs is not directly linked to the expression levels of Esl1-regulated and Esl2-regulated coding genes.

**DISCUSSION**

Here, we have shown that Esl1 and Esl2 share extensive sequence similarity and a similar domain topology with hEST1A/B (SMG5/6). Moreover, given that the “original” yeast hEST1A/B-homolog Est1 lacks the defining PIN domain (Figure 1), Esl1 and Esl2—at least from a structural perspective—may be considered to be the “real” orthologs of hEST1A/B. However, despite these structural similarities, several independent lines of experimental evidence indicated that Esl1 and Esl2 are seemingly neither involved in telomere length and structural maintenance mechanisms nor involved in NMD-related functions in yeast. Instead, the findings that loss of Esl1 and of Esl2 lead to synthetic sickness with two different components of the Rim101 pH-sensing pathway (Figure 4B) and to upregulation or downregulation of glucose and amino acid metabolic genes in the opposite direction as physiological requirements (Table 1 and Figure 5) indicate that these two PIN domain proteins might contribute to environment-sensing adaptive transcriptional response mechanisms.

Dfg16 and Rim8 regulate the proteolytic cleavage of the Rim101 transcription factor under alkaline conditions to facilitate its nuclear translocation and activation of pH-responsive genes (Lamb and Mitchell 2003; Lamb et al. 2001). While our work was in progress, the Rim9, Rim13, and Rim20 genes involved in the Rim101 pH-responsive pathway were identified as genetic interactors of ESL2 in another genome-wide synthetic genetic interaction screen (Costanzo et al. 2010), further supporting that Esl1 and Esl2 may function in a pathway parallel to Rim101. Interestingly, the set of genes regulated in response to alkaline conditions overlaps considerably with the response to low glucose conditions (Ruiz et al. 2008; Serrano et al. 2006; Viladevall et al. 2004), particularly with regard to glucose-repressed genes involved in glucose utilization and carbohydrate metabolism. Consistent with this, a high proportion of deregulated transcripts in

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Figure 4  Genetic interactions of ESL1 and ESL2 with TRF4 and DFG16/RIM8. Tetrad dissection of compound heterozygous diploid strains for esl1Δ and esl2Δ as well as trf4Δ (A) or with rim8Δ or dfg16Δ (B). Genotypes of spores are indicated in (A); circles in (B) denote triple mutants. Plates were incubated for 3–4 days at 30°C.

ADE17, and the cytoplasmic serine hydroxymethyltransferase SHM2—were downregulated, a phenomenon that is usually only observed on withdrawal of glucose from the environment (Subramanian et al. 2005).

Similar aberrations were observed for genes involved in pyridoxine metabolism (SN21 and SNO1), amino acid biosynthesis (ARG3 and CPA2), and the ribosomal protein 18B (RPL18B), which previously have been reported to be coregulated with the genes involved in one-carbon metabolism (Gelling et al. 2004). Taken together, the data indicate that esl1Δ esl2Δ double mutants may have a defect in adapting the expression of hexose and one-carbon metabolism genes to environmentally appropriate requirements.

To determine if Esl1 and Esl2 directly regulate these transcripts via their PIN domains, we performed similar reverse-transcriptase PCR analyses of selected upregulated transcripts using the nuclease-dead mutant alleles. However, in the case of HXT6/HXT7 and HXK1, transcript
**ORF** | **Gene** | **Fold change**
--- | --- | ---
**Transport**
YCR098C | GIT1 | +3.5
YBR286C | PHO89 | +2.7
YKL217W | JEN1 | +2.8
YDR343C | HXT6 | +4.4
YDR342C | HXT7 | +4.0
YEL069C | HXT13 | -2.1
YDR345C | HXT3 | -6.7
**Carbohydrate metabolism**
YFR053C | HXK1 | +3.3
YPK160W | GPH1 | +2.1
YMR105C | PGM2 | +2.0
YBR299W | MAL32 | +2.1
YGR292W | MAL12 | +2.2
YBR297W | MAL33 | +2.1
YJL153C | INO1 | +2.2
YOR178C | GAC1 | +2.5
YER096W | SHC1 | -2.6
**One-carbon metabolism**
YDR019C | GCV1 | -7.3
YMR185W | GCV2 | -6.5
YAL044C | GCV3 | -2.7
YLR056C | SHM2 | -2.3
YMR120C | ADE17 | -2.6
**Pyridoxine metabolism**
YMR096W | SNZ1 | -2.7
YMR095C | SNO1 | -3.2
**Amino acid biosynthesis**
YJL088W | ARG3 | -2.2
YJR109C | CPA2 | -2.7
YIR034C | LYS1 | -2.5
**Translation**
YNL301C | RPL18B | +2.3

**ORF** | **Gene** | **Fold change**
--- | --- | ---
**Transcription**
YIL150C | MCM10 | +2.0
YBR050C | REG2 | +2.3
YER028C | MIG3 | -3.0
**Protein modification**
YLL042C | ATG10 | +2.0
**Aerobic respiration**
YKL093W | MBR1 | +2.0
YMR081C | ISF1 | +2.7
**Sporulation**
YOR242C | SSP2 | -2.0
**Drug resistance**
YDR281C | PHM6 | +2.2
**Stress response**
YBL075C | SSA3 | -2.2
YER103W | SSA4 | -2.0
YCR021C | HSP30 | +3.3
YHR136C | SPL2 | +2.6

**Unclassified**
YDL048C | STP4 | +2.1
YLR327C | TMA10 | +3.0
YER067W | RGI1 | +3.1
YNR034W-A | YHR136C | +2.5
YHR033W | YGR182C | +2.3
YPL014W | YLR297W | +2.2
YGL157W | ARG1 | +2.2
YFL015C | YIL152W | +2.2
YJJ135W | YLR464W | +2.2
YEL076C-A | -2.0

**Figure 5** Classification of deregulated transcripts from the microarray. Gray boxes highlight genes that were expressed at levels that were opposite of what was expected for the glucose and glycine concentrations used.

**esl1Δ esl2Δ** mutants are involved in hexose transport, lactose transport, and carbohydrate metabolism (e.g., HXT13, HXT3, HXT6, HXT7, JEN1, HXK1, PGM2, MAL12, MAL32, and MAL33; Table 1), and should have been repressed under the high glucose conditions during the experiment. At the same time, another set of genes involved in one-carbon metabolism (GCV1, GCV2, GCV3, SHM2, and ADE17) as well as some coregulated transcripts such as genes involved in pyridoxine metabolism (SNZ1 and SNO1) and amino acid biosynthesis (ARG3 and CPA2), which are normally downregulated in response to glycine withdrawal (Gelling et al. 2004; Subramanian et al. 2005), were found to be downregulated under growth conditions with sufficient glycine in the medium (Table 1). Altogether, these findings indicate that **esl1Δ esl2Δ** mutants are defective in sensing or regulating the adaptive response to environmental growth conditions.

**Saccharomyces cerevisiae** contains seven PIN domain–containing proteins: Nob1 and Utp24, which are involved in ribosome biogenesis by assisting in endonucleolytic cleavage of rRNA precursors (Bleichert et al. 2006; Fatica et al. 2003; Fatica et al. 2004); Nmd4, which was isolated in a two-hybrid screen for Upf1-interacting proteins but whose role in NMD remains to be determined (He and Jacobson 1995); Swt1, which has bona fide endoribonuclease activity and contributes to mRNA quality-control at the nuclear pore complex (Rother et al. 2006; Schaeffer et al. 2009; Skrzynz et al. 2009); Rrp44, which forms part of the core of the nuclear and cytoplasmic RNA processing exosome complex and exhibits exoribonuclease activity (Dziembowski et al. 2007; Liu et al. 2006) as well as endoribonuclease activity in vitro (Schaeffer et al. 2009), and the previously uncharacterized related proteins Esl1 and Esl2. Although the conceptual connection to
environment-sensing adaptive response pathways may explain the synthetic sickness of \textit{esl1}Δ \textit{esl2}Δ mutants with \textit{dfg16}Δ and \textit{rim8}Δ (Figure 4B), the reason for their synthetic sickness with \textit{trf4}Δ is less clear (Figure 4A). A surprising finding was that the most highly deregulated transcripts in \textit{esl1}Δ \textit{esl2}Δ mutants were associated with similarly deregulated noncoding RNAs (Figure 6). Because Trf4 is a component of the TRAMP complex involved in the degradation of at least some of these RNAs, a possible explanation could be that the combination of increased expression and reduced degradation of CUTs and SUTs leads to reduced genetic fitness of \textit{esl1}Δ \textit{esl2}Δ \textit{trf4}Δ triple mutants. Similarly, the reasons for increased mitochondrial genome instability and altered drug sensitivities in \textit{esl1} and \textit{esl2} mutants (Figure 2) also remain to be determined. However, mitochondrial DNA integrity is very sensitive to fluctuations in cellular dNTP levels (Zhao \textit{et al.} 1998), and it is conceivable that dNTP homeostasis would be affected by impaired hexose metabolism in \textit{esl1}Δ \textit{esl2}Δ mutants.

Budding yeast are able to proliferate and survive in diverse and often rapidly changing environments—for example, during the fermentation process with quickly changing glucose, ethanol, and acidity levels—but the adaptive response mechanisms remain poorly understood. Our study indicates that the previously uncharacterized \textit{Esl1} and \textit{Esl2} proteins may be involved in this process and provide a basis for future investigations into the detailed mechanisms by which they

\textbf{Figure 6} Gene expression changes in \textit{esl1}Δ \textit{esl2}Δ mutants. (A) Graph showing fold changes of the 10 most highly upregulated and the 10 most highly downregulated transcripts from the microarray. (B and C) Schematic illustration of genomic loci of the top 10 upregulated (B) and top 10 downregulated (C) genes from the microarray (black) with neighboring cryptic unstable transcripts (CUTs; purple) and stable unannotated transcripts (SUTs; orange). (D–F) Reverse-transcriptase polymerase chain reaction (PCR) analysis of the expression levels of various transcripts of the indicated genotypes. Schematic diagrams of the genomic loci of analyzed transcripts are shown above each gel. (G) Western analysis of Hxk1 levels of two independent clones of the indicated genotypes. WT, wild-type.
exert this function as well as investigations into the roles of their nuclease domains in these and other processes.

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