SUPPLEMENTARY DATA

Experimental Procedures

Yeast Strains

All strains used in this study are listed in supplemental Table S1. Strains containing deletions of *EDC3*, *UPF1*, *DCP1*, *XRN1*, *SKI2*, *SKI7*, *CCR4*, or *RRP6* were constructed by gene replacement (Guthrie and Fink, 1991), using DNA fragments harboring the corresponding null alleles. Each genomic DNA deletion was confirmed by PCR analysis. Strains containing deletions of *DHH1*, *PAT1*, *LSM1*, or *LSM7* were purchased from Open Biosystems. Strains harboring the temperature-sensitive *rpb1-1*, *prt1-1*, or *sup45-2* alleles were constructed by the pop-in and pop-out technique (Guthrie and Fink, 1991). Strains harboring the temperature-sensitive *yra1-1* allele were constructed by plasmid shuffling (Guthrie and Fink, 1991).

Plasmids

All plasmids used in this study are listed in Table S2. *YRA1* exon1, intron, or exon2 chimeric alleles were all constructed through *in vivo* recombination in yeast cells as described previously (He et al., 1996). *YRA1* alleles harboring deletions of exon1, intron, or exon2 sequences, or containing insertions of a stem-loop structure, were generated by PCR and molecular cloning. *YRA1* alleles harboring mutations in the *YRA1* translation initiation codon, the 5' splice site, the branch-point region, or the 3' splice site were generated using the QuikChange Site-DirectedMutagenesis Kit (Stratagene). All *YRA1* alleles were confirmed by DNA sequencing.

Oligonucleotides

The oligonucleotides used in this study were obtained from Operon, Inc., and are listed in Table S3.

Cell Growth Conditions

Cells were grown in YPD medium (Microarray analysis and Figures 1, 2, 3B, and 6), or in synthetic complete (SC) medium lacking tryptophan (Figures 3C, 4, 5A, 5B, 5C, and 5D-right side), histidine (Figure 7A and 7C), uracil and tryptophan (Figure 5D-left side), or histidine and tryptophan (Figure 7D and 7E) to select for plasmids. Cultures (10 ml) not involving drug treatment or temperature shifts were grown at 30°C to an OD_{600} of 0.7 and harvested by centrifugation. Cell pellets were frozen on dry ice and then stored at -80°C until RNA was isolated. Cultures involving drug treatment were grown at 30°C to an OD_{600} of 0.7 in a large volume and then concentrated five-fold in the same medium. Where appropriate, the following were added to concentrated cultures: cycloheximide (100 µg/ml), leptomicin (100 ng/ml), or thiolutin (15µg/ml). Drug-treated cells (2ml) were harvested at different time points. For temperature shifts, cells were first grown at 25°C and then treated as described previously (He and Jacobson, 1995).

Microarray Analysis

Five independent expression profiling experiments were carried out for isogenic wild-type (HFY114) and *edc3Δ* (CFY25) strains using Affymetrix Yeast Genome S98 Arrays. Microarray procedures, including RNA isolation, cRNA preparation, microarray hybridization, and data analysis, were as previously described (He et al., 2003), with the

following modifications: First, all microarrays were analyzed with Affymetrix Microarray Suite 5.0 software. Second, a transcript was considered to be differentially expressed if it met two of three previously defined criteria, i.e., it must have an absolute change threshold of 200 units and a change P value ≤0.05. The entire data set can be found at: http://jacobsonlab.umassmed.edu/cgi-bin/pubcontents.cgi?pubcontents=2006-Feng.

Yeast Two-hybrid Analysis

Two-hybrid interactions between full-length Crm1p fused to the *LexA* DNA- binding domain and full-length Edc3p fused to the Gal4p activation domain were assayed as described previously (He et al., 1997).

Results

Identification of Transcripts Differentially Expressed in *edc3Δ* Cells

Five independent expression profiling experiments were carried out with *EDC3* and *edc3Δ* strains and differentially expressed transcripts were initially identified based on three stringent criteria. First, the hybridization signal values of a specific transcript in the wild-type and the *edc3Δ* strains had to have a relative change of at least 2-fold and an absolute change of at least 200 units. Second, these changes had to be reproducible in at least 80% of the independent replicate experiments. Third, these changes had to demonstrate statistically significant P values ≤0.05. To our surprise, this data analysis revealed that, among the 7839 potential transcripts analyzed, only a single transcript

met these criteria. In *edc3Δ* cells, the *EDC3* mRNA itself was decreased more than 10-fold relative to its level in wild-type cells (Table 4S), consistent with the fact that the *edc3Δ* strain harbors a complete *EDC3* deletion. Although this observation validated the overall experiment, we reasoned that our stringent criteria may have overlooked at least two classes of transcripts. For example, highly expressed transcripts are prone to signal saturation and intron-containing transcripts might be missed since the oligonucleotide probes on our arrays do not differentiate intron-containing pre-mRNA signals from mRNA signals. We, therefore, lowered the analysis stringency by eliminating the minimum 2-fold change requirement and reanalyzed our data. This new analysis identified four additional differentially expressed transcripts in the *edc3Δ* strain: two transcripts showed increased expression and two others showed decreased expression (Table 4S). One of the up-regulated transcripts is encoded by the *RPS28B* gene and codes for a 40S ribosomal protein (Lecompte et al., 2002). The other up-regulated transcript is encoded by the *YRA1* gene and codes for an hnRNP-like protein (Yra1p) involved in an early stage of mRNA export (Portman et al., 1997; Strasser and Hurt, 2000). The two down-regulated transcripts, encoded by the *URA1* and *URA4* genes, may well reflect our use of the *URA3* gene as a selectable marker for replacement of the *EDC3* coding region. *URA1*, *URA3*, and *URA4* all code for enzymes involved in uracil biosynthesis (Denis-Duphil, 1989) and the decreased expression of *URA1* and *URA4* transcripts in the *edc3Δ* strain is presumably related to the expression of *URA3*, not to the deletion of *EDC3*.

Inactivation of Yra1p Promotes *YRA1* Pre-mRNA Splicing

To further understand the role of Yra1p in its autoregulation, we examined the effects of mutations in the *YRA1* gene on levels of *YRA1* pre-mRNA and mRNA in an *edc3Δ* background. We reasoned that some loss of function mutations may also fail to autoregulate. We first analyzed *yra1-1*, a ts allele whose encoded protein contains multiple amino acid substitutions and is defective in mRNA export even when cells are grown at room temperature (Strasser and Hurt, 2000). As shown in Figure 2S, when cells were grown at 25°C (t=0) or were shifted to 37°C for 30 min, *edc3Δyra1-1* cells accumulated significantly lower levels of *YRA1* pre-mRNA than *edc3ΔYRA1* cells. In contrast, under these two growth conditions, *edc3Δyra1-1* cells accumulated significantly higher levels of *YRA1* mRNA than *edc3ΔYRA1* cells. The lower *YRA1* pre-mRNA to mRNA ratios in *edc3Δyra1-1* cells indicate that the *yra1-1* allele is defective in autoregulation and suggest that Yra1p regulates its own expression by inhibiting *YRA1* pre-mRNA splicing.

FIGURE 1S

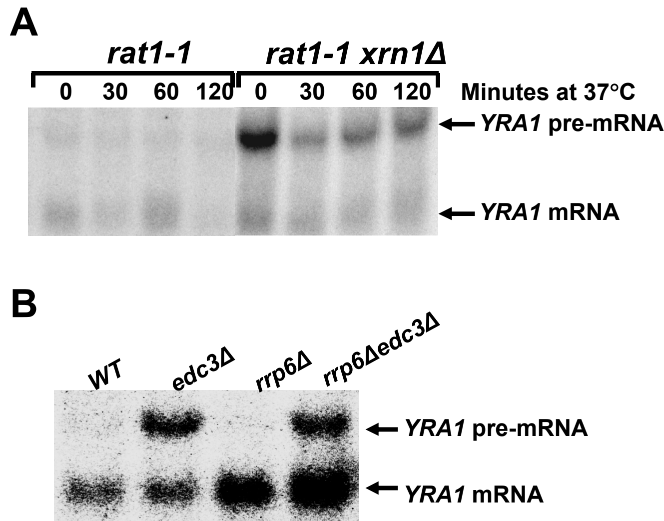

Figure 1S. Inactivation of Ra1t1p or Depletion of Rrp6p Does Not Affect the Accumulation of *YRA1* Pre-mRNA. A. Effect of inactivation of Rat1p on levels of *YRA1* pre-mRNA and mRNA. *Rat1-1* or *rat1-1xrn1Δ* cells were grown in SC minus uracil medium at 25°C and then shifted to 37°C for 30, 60, and 120 min. The levels of *YRA1* pre-mRNA and mRNA were analyzed by northern blotting. B. Effect of depletion of Rrp6 on the levels of *YRA1* pre-mRNA and mRNA. Cells of the indicated genotypes were grown in YEPD medium and the levels of *YRA1* pre-mRNA and mRNA in these cells were analyzed by northern blotting. Note that *rrp6Δ* cells accumulated higher levels of *YRA1* mRNA than *RRP6* cells. The simplest explanation for this observation is that, in wild-type cells, a fraction of the *YRA1* pre-mRNA that is committed to the splicing pathway is degraded by the nuclear exosome.

FIGURE 2S

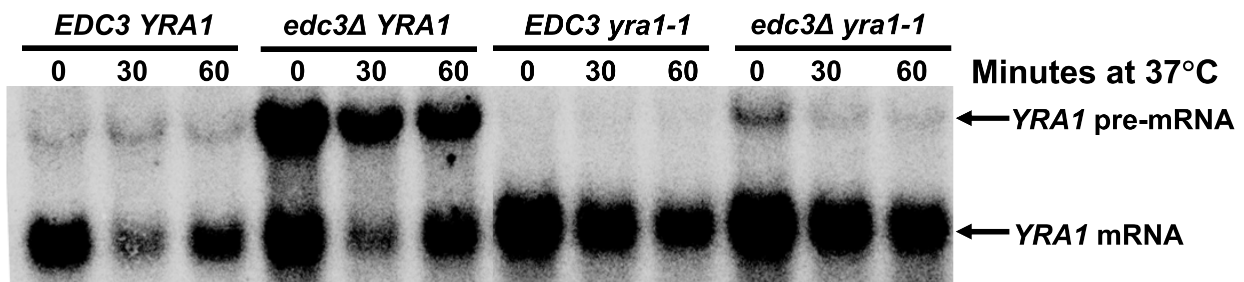

Figure 2S. Inactivation of Yra1p Promotes *YRA1* Pre-mRNA Splicing. Cells of the indicated genotypes were grown in SC minus uracil medium at 25°C and then shifted to 37°C. The levels of *YRA1* pre-mRNA and mRNA were analyzed by northern blotting.

FIGURE 3S

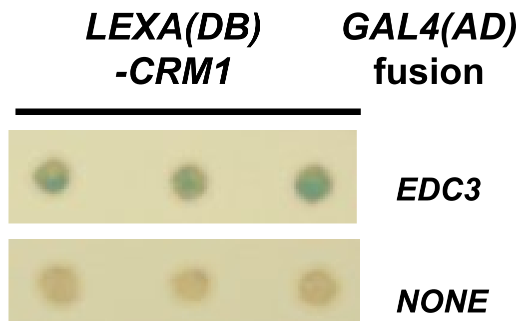

Figure 3S. Crm1p Interacts With Edc3p in the Yeast Two-hybrid System. Yeast plasmids harboring LexA(DB)-*CRM1* and Gal4(AD)-*EDC3* fusions were co-transformed into the L40 tester strain. Transformants were selected and the β-galactosidase activity of individual transformants was assayed on plates containing X-Gal.

Figure 4S

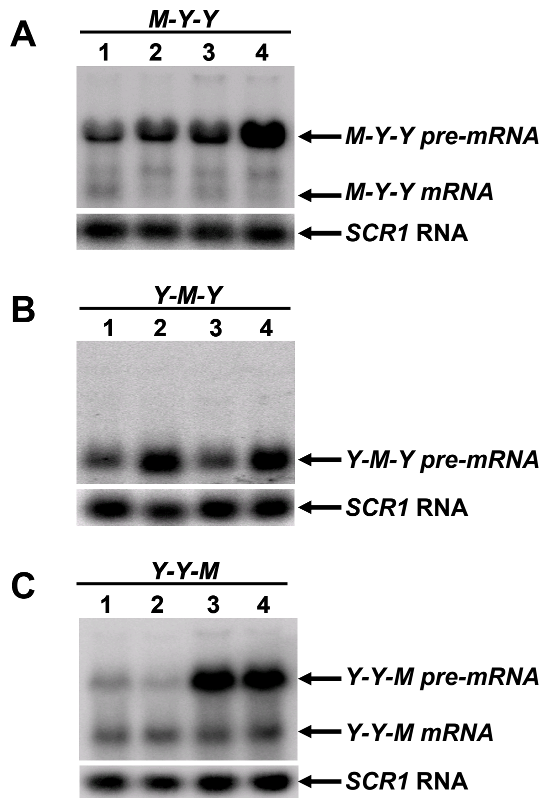

Figure 4S. Cis-acting Determinants of *YRA1* Expression. Alterations of *YRA1* pre-mRNA and mRNA expression mediated by: (A) replacing *YRA1* exon1 with *MER2* exon1, (B) replacing the *YRA1* intron with the *MER2* intron; and (C) replacing *YRA1* exon2 with *MER2* exon2 were analyzed. YCp low-copy plasmids harboring a chimeric allele (depicted above the corresponding blots) were introduced into wild-type (1), *upf1Δ* (2), *edc3Δ* (3), or *upf1Δ edc3Δ* (4) strains and the levels of the respective pre-mRNAs and mRNAs encoded by these alleles were analyzed by northern blotting. Blots A, B, and C were hybridized to *MER2* exon1, intron, or exon2-specific probes, respectively. The positions of chimeric pre-mRNAs and mRNAs are indicated. Y: *YRA1*, and M: *MER2.* Blots were hybridized to a *SCR1* probe to serve as a loading control.

Table 1S. Yeast Strains Used in this study

Strain	Genotype	References
HFY114	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 UPF3*	(He et al., 2003)
HFY871	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 upf1::HIS3 NMD2 UPF3*	(He et al., 2003)
HFY116	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 nmd2::HIS3 UPF3*	(He et al., 2003)
HFY861	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 upf3::HIS3*	(He et al., 2003)
HFY1067	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 UPF3 dcp1::URA3*	(He et al., 2003)
HFY1080	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 UPF3 xrn1::ADE2*	(He et al., 2003)
CFY25	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 UPF3 edc3::URA3*	This study
SYY158	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 UPF3 edc3::URA3 upf1::HIS3*	This study
SYY9	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 UPF3 rpb1-1*	This study
SYY43	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 UPF3 edc3::URA3 rpb1-1*	This study
HFY1170	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 UPF3 ski2::URA3*	This study
SYY17	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 UPF3 ski7::URA3*	This study
SYY21	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 UPF3 ski2::URA3 ski7::ADE2*	This study
CFY13	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 UPF3 prt1-1*	This study
HFY1218	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 UPF3 sup45-2*	This study
SYY60	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 UPF3 edc3::URA3 xrn1::ADE2*	This study
SYY110	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 UPF3 ccr4::LEU2*	This study
SYY114	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 UPF3 edc3::URA3 ccr4::LEU2*	This study
DAt1-1	*MATa ura3-52 trp1-D63 leu2-D1 rat1-1*	(He and Jacobson, 2001)
HFY1102	*MATa ura3-52 trp1-D63 leu2-D1 rat1-1 xrn1::URA3*	(He and Jacobson, 2001)
SYY573	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 UPF3 rrp6::LEU2*	This study
SYY577	*MATa ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 UPF3 edc3::URA3 rrp6::LEU2*	This study
BY4741	*MATa hisΔ1 leu2Δ0 met15Δ0 ura3Δ0*	open biosystems
SYY160	*MATa hisΔ1 leu2Δ0 met15Δ0 ura3Δ0 edc3::URA3*	This study
BY1301	*MATa hisΔ1 leu2Δ0 met15Δ0 ura3Δ0 lsm1::kanMX*	open biosystems
SYY176	*MATa hisΔ1 leu2Δ0 met15Δ0 ura3Δ0 lsm1::kanMX edc3::URA3*	This study
BY7383	*MATa hisΔ1 leu2Δ0 met15Δ0 ura3Δ0 lsm7::kanMX*	open biosystems
SYY172	*MATa hisΔ1 leu2Δ0 met15Δ0 ura3Δ0 lsm7::kanMX edc3::URA3*	This study
BY5797	*MATa hisΔ1 leu2Δ0 met15Δ0 ura3Δ0 pat1::kanMX*	open biosystems
SYY164	*MATa hisΔ1 leu2Δ0 met15Δ0 ura3Δ0 pat1::kanMX edc3::URA3*	This study
BY3858	*MATa hisΔ1 leu2Δ0 met15Δ0 ura3Δ0 dhh1::kanMX*	open biosystems
SYY214	*MATa hisΔ1 leu2Δ0 met15Δ0 ura3Δ0 dhh1::kanMX edc3::URA3*	This study
yra1 shuffle	*MATa ade2 his3 leu2 trp1 ura3 yra1::HIS3 pRS316-YRA1*	(Strasser and Hurt, 2000)
SYY88	*MATa ade2 his3 leu2 trp1 ura3 yra1::HIS3 pRS314-yra1-1*	This study
SYY131	*MATa ade2 his3 leu2 trp1 ura3 yra1::HIS3 pRS314-yra1-1 edc3::URA3*	This study
SYY529	*MATa ade2 his3 leu2 trp1 ura3 yra1::HIS3 pRS316-YRA1 edc3::LEU2*	This study
FSY1135	*MATa ade2 his3 leu2 trp1 ura3 yra1::HIS3 yra2::kan pRS316-YRA1*	(Zenklusen et al., 2001)
SYY606	*MATa ade2 his3 leu2 trp1 ura3 yra1::HIS3 yra2::kan yeplac112-YRA2*	This study
SYY621	*MATa ade2 his3 leu2 trp1 ura3 yra1::HIS3 yra2::kan yeplac112-YRA2 edc3::LEU2*	This study
MNY12	*MATa his3 leu2 trp1 ura3 crm1::kanR pRS313-GFP-crm1(T539C)*	(Neville and Rosbash, 1999)
SYY614	*MATa his3 leu2 trp1 ura3 crm1::kanR pRS313-GFP-crm1(T539C) edc3::URA3*	This study
SYY834	*MATa his3 leu2 trp1 ura3 crm1::kanR pRS313-GFP-crm1(T539C) rrp6::LEU2*	This study
SYY838	*MATa his3 leu2 trp1 ura3 crm1::kanR pRS313-GFP-crm1(T539C) edc3::URA3 rrp6::LEU2*	This study
SYY571	*MATa or α ade2 his3 15 leu2 trp1 ura3 mex67::HIS3 pRS316-URA3-MEX67*	From Dr. Rosbash
SYY572	*MATa ade2 his3 15 leu2 trp1 ura3 mex67::HIS3 pRS314-mex67-5*	From Dr. Rosbash
SYY1902	*MATa or α ade2 his3 15 leu2 trp1 ura3 mex67::HIS3 edc3::LEU2 pRS316-URA3-MEX67*	This study
SYY605	*MATa ade2 his3 15 leu2 trp1 ura3 mex67::HIS3 edc3::LEU2 pRS314-mex67-5*	This study
SYY749	*MATa ade2 his3 15 leu2 trp1 ura3 mex67::HIS3 edc3::LEU2 upf1::URA3 pRS314-mex67-5*	This study
SYY1913	*MATa ade2 his3 15 leu2 trp1 ura3 mex67::HIS3 edc3::LEU2 dcp1::ADE2 pRS314-mex67-5*	This study
SYY1920	*MATa ade2 his3 15 leu2 trp1 ura3 mex67::HIS3 edc3::LEU2 xrn1::ADE2 pRS314-mex67-5*	This study
L40	*MATa ade2 his3D200 leu2-3,112 trp1-901 LYS2::(lexAop)4-HIS3 URA3::(lexAop)8lacZ gal4 gal80*	Stanley Hollenberg

Table 2S. Plasmids used in this study

Name	Allele	Description
SYE22	pRS406*-rpb1-1(-XbaI)	Contains the ts rpb1-1allele as an 4.0 kb EcoRI-HindIII fragment
SYE26	Bs-ski7::URA3	Contains the ski7::URA3 null allele as a NotI-SalI fragment
SYE28	Bs-ski7::ADE2	Contains the ski7::ADE2 null allele as a NotI-SalI fragment
SYE200	Bs-edc3::LEU2	Contains the edc3::LEU2 null allele as a NotI-SalI fragment
CFE7	Bs-edc3::URA3	Contains the edc3::URA3 null allele as a NotI-SalI fragment
HFE1397	Bs-upf1-1::URA3	Contains the upf1::URA3 null allele as a NotI-SalI fragment
	Bs-rrp6::LEU2	Contains the rrp6::LEU2 null allele as a HindIII-BamHI fragment
SYE44 (PIA278-1)	pRS314-YRA1	Contains wild-type YRA1 allele as a 2.0 kb BamHI-XhoI fragment
SYE46(PIA286-1)	pRS314-YRA1-ΔIVS	Same as SYE44 but lacks the *YRA1* intron
SYE58	pRS316-YRA1	Contains wild-type YRA1 allele as a 3.0 kb HindIII-SacI fragment
SYE60	pRS314-yra1-1	Contains the yra1-1 allele as a 3.0 kb HindIII-SacI fragment
SYE181(pFS2261)	YepLac112-Myc-YRA2	Contains Myc-tagged *YRA2* allele as a 1.7 kb BamHI-BamHI fragment
SYE70	pRS316-YRA1 (HindIII-SacI)	Contains wild-typ YRA1 allele as a 2.2 Kb HindIII-SacI fragment
SYE74	pRS314-YRA1(XhoI-SacI)	Contains wild-typ YRA1 allele as a 2.2 Kb XhoI-SacI fragment
SYE80	pRS314-YRA1-C-Y-Y	Same as in SYE74 but the 382-nt YRA1exon 1 was replaced by the 83-nt CYH2 exon1
SYE82	pRS314-YRA1-M-Y-Y	Same as in SYE74 but the 382-nt YRA1exon 1was replaced by the 385-nt MER2 exon1
SYE507	pRS314-YRA1-H-Y-Y	Same as in SYE74 but the 285-nt coding region of YRA1exon 1was replaced by the 663-nt HIS3 coding re
SYE474	pRS314-YRA1-exon1-reverse	Same as in SYE74 but the last 286 nts of YRA1exon 1 was replaced by its complementay sequences
SYE84	pRS314-YRA1-Y-C-Y	Same as in SYE74 but the 766-nt YRA1 intron was replaced by the 511-nt CYH2 intron
SYE87	pRS314-YRA1-Y-M-Y	Same as in SYE74 but the 766-nt YRA1 intronwas replaced by the 80-nt MER2 intron
SYE90	pRS314-YRA1-Y-R-Y	Same as in SYE74 but the 766-nt YRA1 intronwas replaced by the 398-nt RPS51A intron
SYE92	pRS314-YRA1-Y-Y-C	Same as in SYE74 but the 588-nt YRA1 exon 2 was replaced by the 485-nt CYH2 exon2
SYE92	pRS314-YRA1-Y-Y-M	Same as in SYE74 but the 588-nt YRA1 exon2 was replaced by the 827-nt MER2 exon2
SYE93	pRS314-YRA1-Y-Y-R	Same as in SYE74 but the 588-nt YRA1 exon2 was replaced by the 529-nt RPS51A exon2
SYE73	pRS314-YRA1-N84	Same as in SYE74 but contains an internal deletion from A.A. 2 to 85 in exon1
SYE137	pRS314-YRA1-DE-CI10	Same as in SYE74 but contains an internal deletion from A.A. 11 to 85 in exon1
SYE139	pRS314-YRA1-DE-CI20	Same as in SYE74 but contains an internal deletion from A.A. 21 to 85 in exon1
SYE141	pRS314-YRA1-DE-CI30	Same as in SYE74 but contains an internal deletion from A.A. 31 to 85 in exon1
SYE143	pRS314-YRA1-DE-CI40	Same as in SYE74 but contains an internal deletion from A.A. 41 to 85 in exon1
SYE145	pRS314-YRA1-DE-CI50	Same as in SYE74 but contains an internal deletion from A.A. 51 to 85 in exon1
SYE147	pRS314-YRA1-DE-CI60	Same as in SYE74 but contains an internal deletion from A.A. 61 to 85 in exon1
SYE149	pRS314-YRA1-DE-CI70	Same as in SYE74 but contains an internal deletion from A.A. 71 to 85 in exon1
SYE151	pRS314-YRA1-DE-CI80	Same as in SYE74 but contains an internal deletion from A.A. 81 to 85 in exon1
SYE374	pRS314-YRA1-DI-R1-F7	Same as SYE74 but contains a 461-nt internal deletion from nts 27 to 487 of YRA1 intron
SYE78	pRS314-YRA1-DE2	Same as SYE74 but contains a deletion of first 351nts of YRA1exon2
SYE160	pRS314-YRA1-AUA	Same as in SYE74 but contains AUG to AUA mutation in the transaltion initiation codon
SYE161	pRS314-YRA1-AUC	Same as in SYE74 but contains AUG to AUC mutation in the transaltion initiation codon
SYE163	pRS314-YRA1-AUU	Same as in SYE74 but contains AUG to AUU mutation in the transaltion initiation codon
SYE240	pRS316-YRA1-AUA	Same as in SYE74 but contains AUG to AUA mutation in the transaltion initiation codon
SYE298	pRS314-YRA1-m5SS	Same as in SYE74 but contains GUAUGU to GUAUAU mutation in the 5' splicing site
SYE300	pRS314-YRA1-m3SS	Same as in SYE74 but contains AG to UC mutation in the 3' splicing site
SYE302	pRS314-YRA1-mBB2	Same as in SYE74 but contains UGACUAAC to UGACUACC mutation in the branchpoint region
SYE415	pRS314-YRA1-N84-m5SS	Same as in SYE73 but contains GUAUGU to GUAUAU mutation in the 5' splicing site
SYE417	pRS314-YRA1-N84-m3SS	Same as in SYE73 but contains AG to UC mutation in the 3' splicing site
SYE419	pRS314-YRA1-N84-mBB2	Same as in SYE73 but contains UGACUAAC to UGACUACC mutation in the branchpoint region
SYE515	pRS316-YRA1-AUA-m5SS	Same as in SYE240 but contains GUAUGU to GUAUAU mutation in the 5' splicing site
SYE517	pRS316-YRA1-AUA-m3SS	Same as in SYE240 but contains AG to UC mutation in the 3' splicing site
SYE519	pRS316-YRA1-AUA-mBB2	Same as in SYE240 but contains UGACUAAC to UGACUACC mutation in the branchpoint region
SYE234	pGAD-C2-EDC3-FL	Contains the entire EDC3 coding region as an EcoR-SalI fragment
SYE324	pBTM116-CRM1-FL	Contains the entire CRM1 coding region as an EcoR-SalI fragment

Table 3S. Oligonucleotides used in this study

Name	Sequences
YRA1-1	AACCAAAGAGAGAAAAGCCTGCTAA
YRA1-2	GATCGAGCTCTGAGGACCATCAATTAGTAAG
YRA1-3	ACTTGAAGAACTATAAAAGGCCGC
YRA1-4	GTGTGCCATATCCTTCCTTACAAA 3'
YRA1-5	GTATGTTAATACGTGAAATGAGAGCT
YRA1-6	GAGTTGCCAAGCTCTTGGACACCACTA
YRA1-5-R	TTCGTCTAAGGATTTATCTAAGTTAGC
YRA1-7-R	GCAGATGTAGGTATTTTCTTAATATGG
Y-C-1A	TCGTGTGTTTAGTAGTAGGTTTTTTTGTTAGAAGAAGTTTATTAAATCCCAGAACAATCATCCAA
Y-C-1B	GTAACTAAACAAATAGCTCTCATTTCACGTATTAACATACTGAGACGTGACCTCTGTGCTTTCTA
Y-C-2A	CGAAGGTTTGCCAAGGGACATTAAGCAGGATGCTGTAAGAGTATGTAGTTCCATTTGGAAGAGGG
Y-C-2B	AAACTCTTTGAACACCACCTACTTGAGATGCAAAAAATTCCTGTACAAAAAAAATATTGTAATGA
Y-C-3A	TCATAGAGATATATGACTAACTTTTTTTTTTTTATTATAGGGTAAAGGTCGTATCGGTAAGCACA
Y-C-3B	GACATATTATGAGTCAAATATGCCGAATAAACTTTAAAAGGAAATAATACAGAAGTAGATGTTGA
Y-M-1A	TCGTGTGTTTAGTAGTAGGTTTTTTTGTTAGAAGAAGTTTTCAACAAGAACAGAAAGAAGGAAAA
Y-M-1B	GTAACTAAACAAATAGCTCTCATTTCACGTATTAACATACGTTCGTAGCAGCATCTTGTTCCAGT
Y-M-2A	CGAAGGTTTGCCAAGGGACATTAAGCAGGATGCTGTAAGAGTTCGTACCAACACAGTGCATACCC
Y-M-2B	AAACTCTTTGAACACCACCTACTTGAGATGCAAAAAATTCCTATACTACAGTTGTTAGTAAATGT
Y-M-3A	TCATAGAGATATATGACTAACTTTTTTTTTTTTATTATAGAACGTGAAAACCTTAATAAAGGATT
Y-M-3B	GACATATTATGAGTCAAATATGCCGAATAAACTTTAAAAGCAACTGGCGTGGTTTTTCATTGTAG
Y-R-2A	CGAAGGTTTGCCAAGGGACATTAAGCAGGATGCTGTAAGAGTATGTTAATATGGACTAAAGGAGG
Y-R-2B	AAACTCTTTGAACACCACCTACTTGAGATGCAAAAAATTCCTATTAAAATGACGAAAAGCAATAC
Y-R-3A	TCATAGAGATATATGACTAACTTTTTTTTTTTTATTATAGGGTAGAGTTAGAACCAAGACCGTCA
Y-R-3B	GACATATTATGAGTCAAATATGCCGAATAAACTTTAAAAGAATTAGTAAATATAATAATATTTTT
Y-H-F1	TTTAAGAAATCCATATTAAGAAAATACCTACATCTGCTAAATGACAGAGCAGAAAGCCCTAGTAA
Y-H-R1	GTAACTAAACAAATAGCTCTCATTTCACGTATTAACATACCTACATAAGAACACCTTTGGTGGAG
YRA1-Ex1R-F	CTTTAAGAAATCCATATTAAGAAAATACCTACATCTGCTATCTTACAGCATCCTGCTTAATGTCC
YRA1-Ex1R-R	GTAACTAAACAAATAGCTCTCATTTCACGTATTAACATACAATGTCTGCTAACTTAGATAAATCC
YRA1-DE1-5'	GATCCTCGAGGGTATATTAAAGCTATTTTACCACT
YRA1-DE1-3'	GATCCCTTGGCATTTAGCAGATGTAGGTATTTTCT
YRA1-CI10	GATCCCTTGGGTCTAAGGATTTATCTAAGTTAGCA
YRA1-CI20	GATCCCTTGGACTTCCTGCTTTGTTAGAGCCAATG
YRA1-CI30	GATCCCTTGGACCACGAGTACCACCGACACGGGCT
YRA1-CI40	GATCCCTTGGAACTTGCTTACCAACTCTTCTTGGA
YRA1-CI50	GATCCCTTGGTCTGTTTGGAAGGCTCCTACGTTGG
YRA1-CI60	GATCCCTTGGTGCCCTAGTATTTTTTCTGATAGGG
YRA1-CI70	GATCCCTTGGCTTGGCAACTCTAGCGACTGCGTTT
YRA1-CI80	GATCCCTTGGGACCTTGACCTCTCTAGTGGTGTCC
YRA1-DI-3'	AATCTTGATCTGCCTCCATCGATTG
YRA1-DI-F7	GATCGAATTCTAGCTATGGGAAACATGTCTTTCAT
YRA1-DI-R1	GATCGAATTCAGCTCTCATTTCACGTATTAACATA
YRA1-DE2-5'	TGATCAAGAATTCTCTTTTTAGAGA
YRA1-DE2-3'	GATCAGATCTTCCTATAATAAAAAAAAAAAAGTTA
YRA1-mAUU-F	AATACCTACATCTGCTAAATTTCTGCTAACTTAGATAAATC

YRA1-mAUU-R	GATTTATCTAAGTTAGCAGAAATTTAGCAGATGTAGGTATT
YRA1-mAUC-F	AATACCTACATCTGCTAAATCTCTGCTAACTTAGATAAATC
YRA1-mAUC-R	GATTTATCTAAGTTAGCAGAGATTTAGCAGATGTAGGTATT
YRA1-mAUA-F	AATACCTACATCTGCTAAATATCTGCTAACTTAGATAAATC
YRA1-mAUA-R	GATTTATCTAAGTTAGCAGATATTTAGCAGATGTAGGTATT
YRA1-m5SS-F	GCAGGATGCTGTAAGAGTATATTAATACGTGAAATGAGAGC
YRA1-m5SS-R	GCTCTCATTTCACGTATTAATATACTCTTACAGCATCCTGC
YRA1-mBB2-F	ATCATAGAGATATATGACTACCTTTTTTTTTTTTATTATAG
YRA1-mBB2-R	CTATAATAAAAAAAAAAAAGGTAGTCATATATCTCTATGAT
YRA1-m3SS-F	AACTTTTTTTTTTTTATTATTCGAATTTTTTGCATCTCAAGT
YRA1-m3SS-R	ACTTGAGATGCAAAAAATTCGAATAATAAAAAAAAAAAAGTT
yra1-2-r	CGCCATTTCCTTGTCCAGATCTTC
YRA1-m1	GATGCAAAAAATTCTCTTACAGCATCC
YRA1-p1	AGCTCTCATTTCACGTATTAACATAC
YRA1-D-3UTR-1	CATCGCACTGTAAATAGTGACATATTATGA
YRA1-D-3UTR-2	ACGAGTAACACACGTTTAATCAACCTATCC
YRA1-1-r	TCGAGGCGACACAATACGCCAGCT
YRA1-IN-1	AAACTCGAGAGAGGTCAAGGTCAACGTCGAAGG
YRA1-IN-2	GGGAAGCTTGTTATAACTCAACAAAACTCTTTG
YRA1-DI-5'	GATCGAATTCCTCCTTGATTGTTTGTGTATTGTCC
YEL015W-1	AATTGCGGCCGCGGTCAACAGGTTGCTCGAAAAGAAGCA
YEL015W-2	AGAGGATCCTATGGTTTCTTTTACGAATTACTGTATTGT
EDC3-DS-2A	GATCGGATCCTCTAGATATGGTTTCTTTTACGAATTACTGTATTG
YEL015W-3	AGAGGATCCAACATCGATACCAAGAATTACTTTAGCCTAAACTGGATA
EDC3-DS-3A	GATCGGATCCCTCGAGACCAAGAATTACTTTAGCCTAAACTGGAT
YEL015W-4	AATTGTCGACGCTCAGTTCCTGTCTTCGTAGGATTGG
CRM1-TH-F1	GATCGAATTCATGGAAGGAATTTTGGATTTTTCTAACGAC
CRM1-TH-C1	GATCGTCGACCTAATCATCAAGTTCGGAAGGTTTTAATAA
CRM1-F-S1	AGTTGAGCCATTATTGAACGCTGT
CRM1-F-S2	AGGCAAATCGATGGTTCCGAATGG
CRM1-R-S1	GTCCAAAGTGCATTCAAATACACTC
DBP2-2-r	CTCTGTTACCCCAGCCACCATCTC
RPS28B	CGCAAACGACGAGCTTCACGTTCA
MER2-P	TGAGGGTATGCACTGTGTTGGTACGAAC
MER2-exon1-F	ATGGTCGCTAGAGGTAGAACAGACGAGA
MER2-exon1-R	CGTTCGTAGCAGCATCTTGTTCCAGTAG
MER2-exon2-F	GAAACGTGAAAACCTTAATAAAGGATTT
MER2-exon2-R	TCACAGCTCAGATTCCAGAGTGTCGGGT
18S	CATGGCTTAATCTTTGAGAC
RRP6-1	CCCGGAATTCCCAAAAATATGAGGGCATCGG
RRP6-2	CTAGTCTAGATTCGAGCGAAGTATAATCCTGC

Table 4S. Transcripts Differentially Expressed in *edc3Δ* Cells

Probe set	ORF	Gene	A-WT Signal	A-edc3 Signal	B-WT Signal	B-edc3 Signal	C-WT Signal	C-edc3 Signal	E-WT Signal	E-edc3 Signal	G-WT Signal	G-edc3 Signal
6078_at	YDR381W	YRA1	622.5	944.9	801.4	1092	644.3	1038.9	568.4	1010.9	568.3	961.1
6079_at	YDR381W	YRA1	585.1	1158	561.2	944.7	645.4	1040.5	593.6	1165.5	578.5	1209.1
10069_f_at	YLR264W	RPS28B	2214	2722.5	2219.4	2966.9	2221.2	2744.2	2090.5	3027.8	2013.6	2799.1
10068_i_at	YLR264W	RPS28B	4649.6	4855.4	4040.4	4755.4	4869.1	5681.9	4692.4	6727.4	4337.1	5260.2
5732_at	YEL015W	EDC3	473.6	10	480.5	10	466.8	10	465.4	10	456.8	10
10796_at	YKL216W	URA1	1838.5	786.6	1963.9	1019	1805.3	831.3	1649.7	827.3	1620.7	857.9
9911_at	YLR420W	URA4	790.5	544.9	826.8	577.2	780.1	574.8	741.1	466.5	750.2	471.1

Five independent expression profiling experiments were carried out with wild-type (*EDC3*) and *edc3Δ* strains. Our data analysis indicates that only five transcripts (represented by seven probe sets) are differentially expressed in the *edc3Δ* strain. The raw signal values of each of these probe sets in different experiments (A, B, C, E, and G) are shown in the table.

Table 5S. Micorarray Data

This table is located at

http://jacobsonlab.umassmed.edu/cgi-bin/pubcontents.cgi?pubcontents=2006-Feng.

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