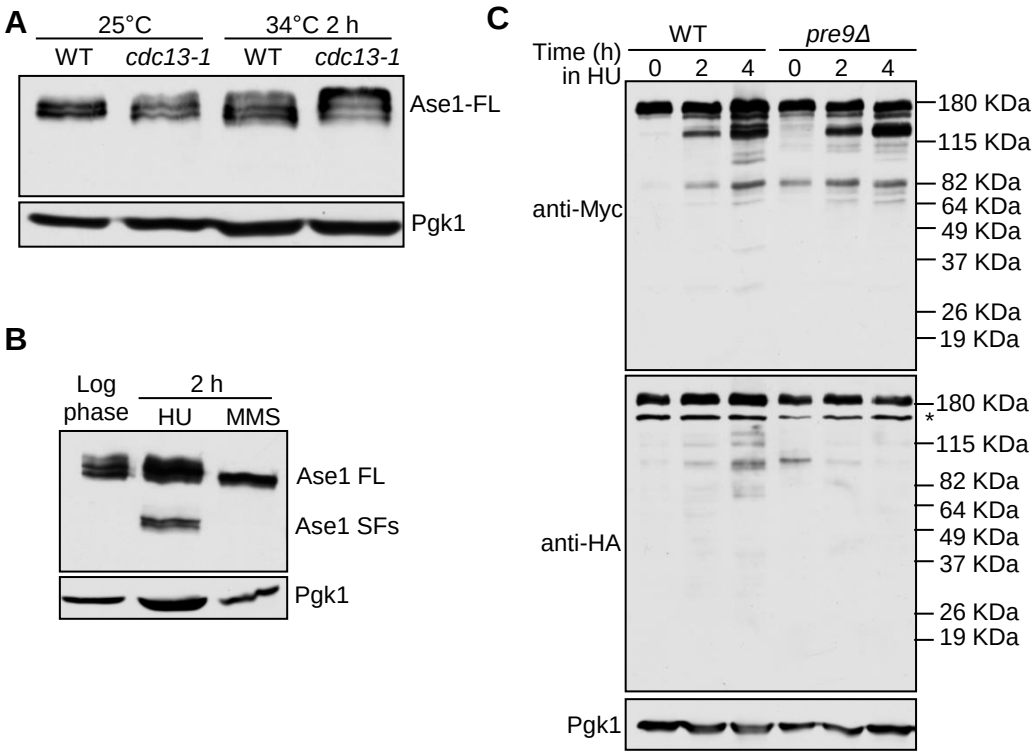
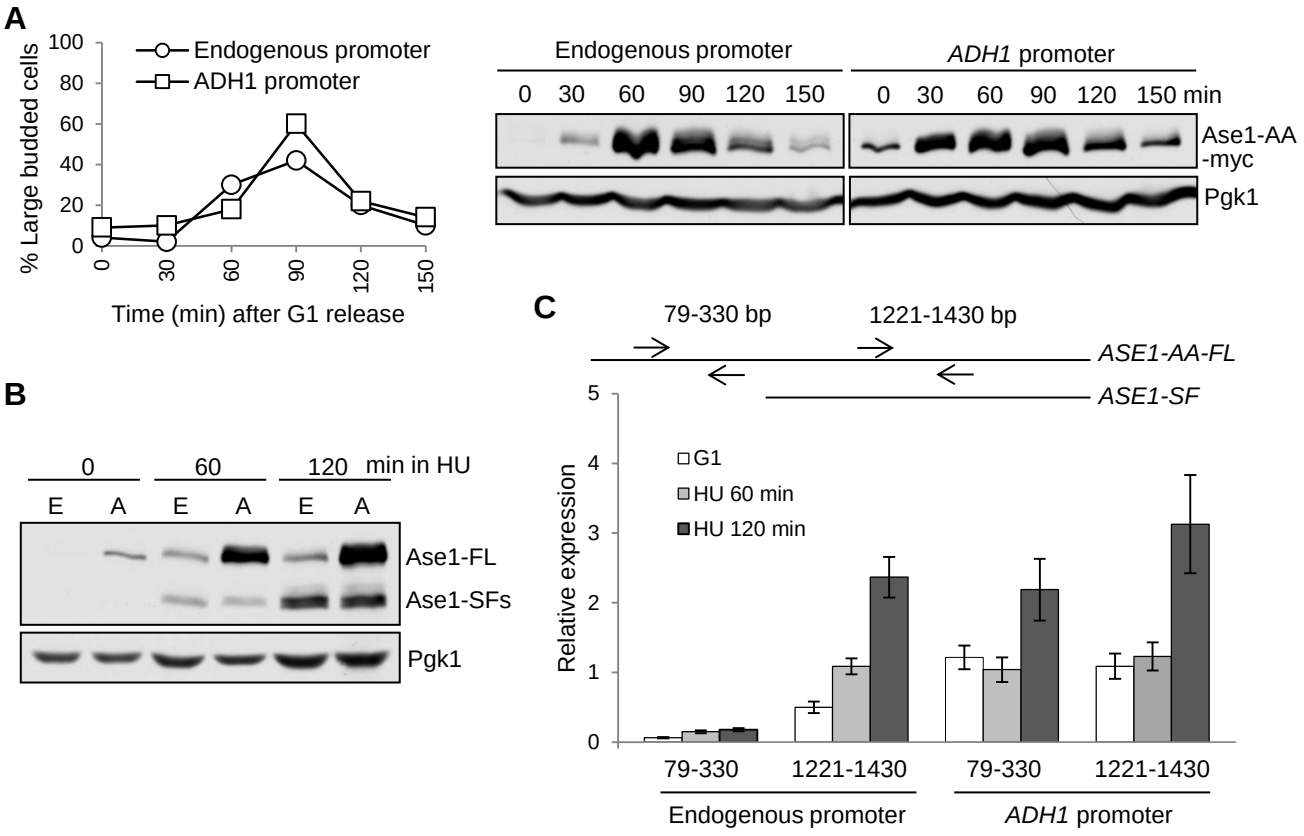
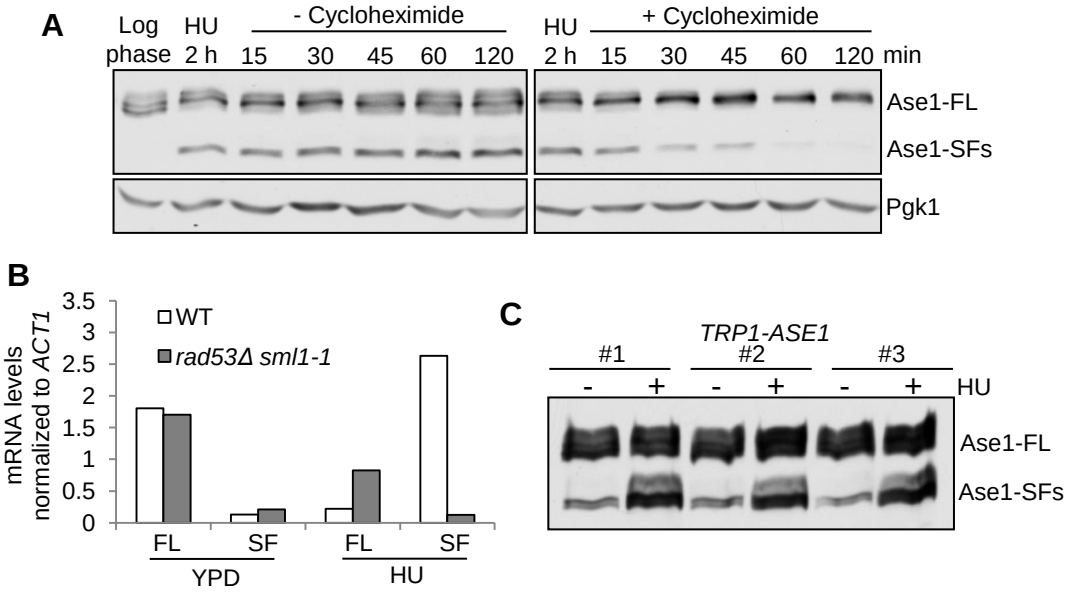
McKnight – Fig. S1

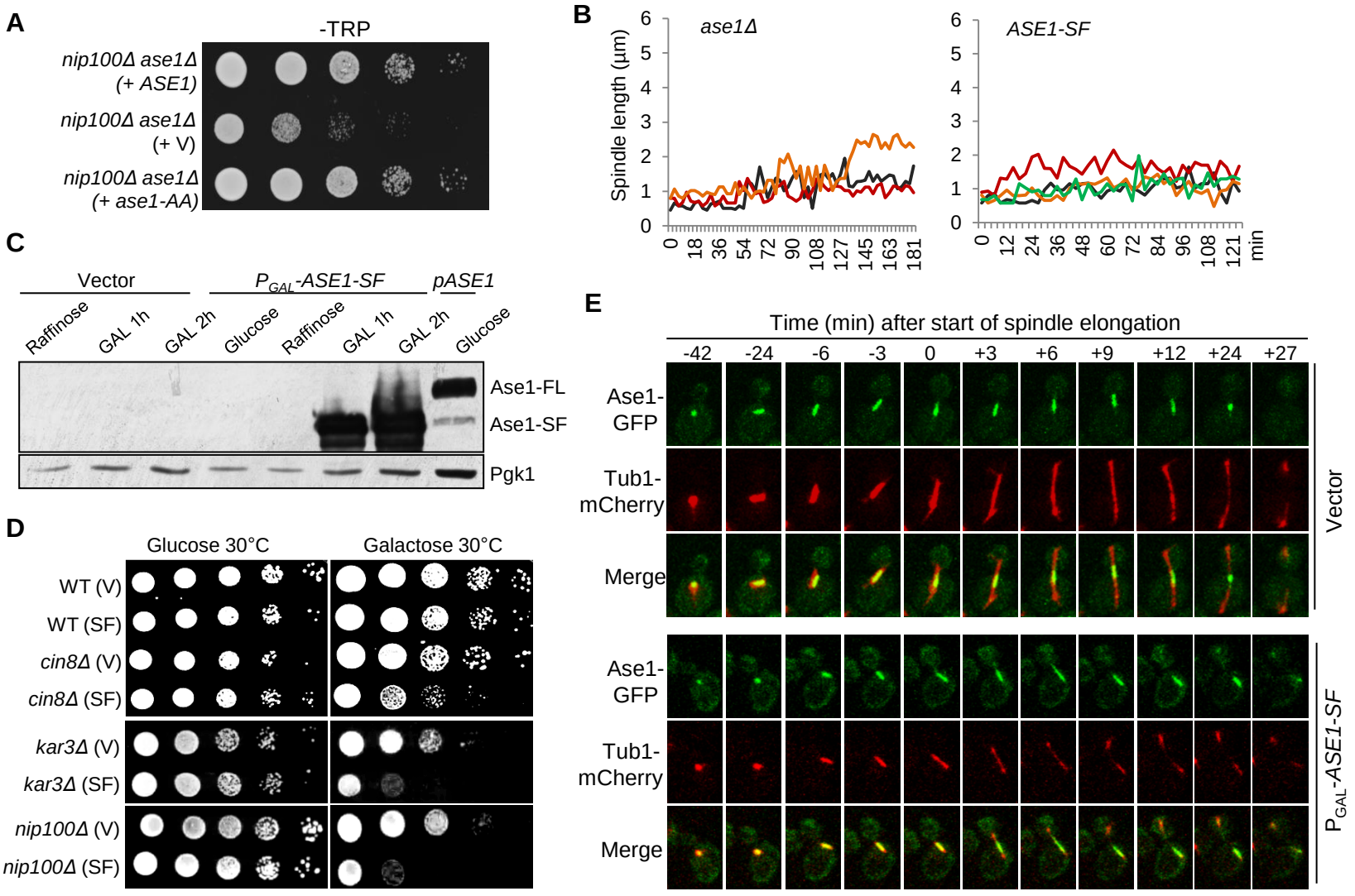


McKnight – Fig. S2



McKnight – Fig. S3




A
-TRP
nip100Δ ase1Δ (+ ASE1)
nip100Δ ase1Δ (+ V)
nip100Δ ase1Δ (+ ase1-AA)
B
ase1Δ
ASE1-SF
Spindle length (µm)
min
C
Vector
PGAL-ASE1-SF
pASE1
Raffinose
GAL 1h
GAL 2h
Glucose
Ase1-FL
Ase1-SF
Pgk1
D
Glucose 30°C
Galactose 30°C
WT (V)
WT (SF)
cin8Δ (V)
cin8Δ (SF)
kar3Δ (V)
kar3Δ (SF)
nip100Δ (V)
nip100Δ (SF)
E
Time (min) after start of spindle elongation
-42
-24
-6
-3
0
+3
+6
+9
+12
+24
+27
Ase1-GFP
Tub1-mCherry
Merge
Vector
PGAL-ASE1-SF

Supplemental Information

Figure legends for supplemental figures

Figure S1. Ase1 short protein fragment expression is HU-specific and not a cleavage product. Related to Figure 1. **(A)** Ase1 short isoforms were not detected in *cdc13-1*-arrested cells. *ASE1-13myc* and *cdc13-1 ASE1-13myc* cells were grown to log phase at 25°C and released to 34°C. Cells were collected at the indicated time points and protein samples were prepared for western blotting. Pgk1 protein levels are shown as a loading control. **(B)** Ase1 short isoforms were not detected in cells treated with 0.033% MMS. *ASE1-13myc* cells were grown to log phase at 30°C, followed by release into medium containing 200 mM HU or 0.033% MMS for 2 hrs. Protein samples were prepared for western blotting before and after treatment. Pgk1 protein levels are shown as a loading control. **(C)** N-terminal cleavage fragments were not detected in *pre9Δ* mutants treated with HU. WT and *pre9Δ* cells containing *3HA-ASE1-13myc* plasmid were grown to log phase at 25°C. The cells were then shifted to 34°C to decrease protein degradation and 200 mM HU was added. Protein samples were prepared at the indicated time points. Western blotting with anti-HA and anti-myc antibodies was used to determine the expression of full length (Ase1-FL) and short isoforms (Ase1-SF) of Ase1 protein. Pgk1 protein levels are shown as a loading control. Asterisk indicates a non-specific band.

Figure S2. Short *ASE1* mRNA expression is independent of full length *ASE1*. Related to Figure 2A-B. **(A)** Expression of *ase1-AA-13myc* under the control of the *ADH1* promoter is increased throughout the cell cycle compared to the endogenous promoter. G_1-arrested *ase1Δ* cells containing *ase1-AA-13myc* plasmid or P_{ADH1}-*ase1-AA-13myc* plasmid were released to fresh media and α-factor was restored to block the second round of cell cycle. Cells were collected at the indicated time points to detect the expression of Ase1-AA protein using western

blotting. Budding index was used to indicate cell cycle stage and Pgk1 protein levels are shown as a loading control. **(B)** The levels of Ase1 short protein isoforms are unaffected by increased full length Ase1. *ase1Δ* cells containing *ase1-AA-13myc* and *ASE1-SF-13myc* plasmids or P_{ADH1}-*ase1-AA-13myc* and *ASE1-SF-13myc* plasmids were grown to log phase and released to medium containing 200 mM HU. Protein samples were prepared for western blotting at the indicated time points. Pgk1 protein levels are shown as a loading control. E: endogenous promoter; A: *ADH1* promoter. **(C)** Constitutive expression of full-length *ase1-AA* mRNA from the *ADH1* promoter does not decrease expression of the short *ASE1* mRNA isoform. (Top) Schematic of *ASE1-FL* and *ASE1-SF* regions. Arrows mark the location of the primers used. (Bottom) RT-qPCR results for transcription derived from the indicated regions of *ASE1*. qPCR for one biological replicate was performed in triplicate and normalized against *ACT1*. Error bars = s.d.

Figure S3. Ase1-SF protein is unstable but expression is likely independent of replication fork stalling. Related to Figure 3. **(A)** Ase1 short protein isoforms are unstable. *ASE1-13myc* cells were grown to log phase at 30°C, followed by release to medium containing 200 mM HU for 2 hrs. Cycloheximide was added to half of the cell culture at 35 μg/mL. Protein samples were prepared for western blotting at the indicated time points. Pgk1 protein levels are shown as a loading control. **(B)** Expression of the short *ASE1* mRNA depends on Rad53. *ASE1* mRNA isoform levels from the northern blotting in Figure 3C were quantified using Image J software. **(C)** Ase1-SF expression is still induced during replication stress when the nearby replication origin is deleted from the genome. The 1500 bp genomic segment upstream of the *ASE1* gene, which includes replication origin *ARS1501*, was replaced with the *TRP1* marker. The resulting three *TRP1-ASE1* strains were grown to log phase and released to YPD or YPD containing 200

mM HU for 2 hrs. Cells were collected and protein samples were prepared for western blotting to determine the expression of Ase1-FL and Ase1-SFs.

Figure S4. Spindle function of Ase1-SF. Related to Figure 4. **(A)** *ase1-AA* mutants can restore Ase1 protein function. Cell cultures with indicated genotypes in stationary phase were 10-fold serial diluted and spotted onto TRP dropout plates and incubated at 30°C for 2 days before scanning. **(B)** *ase1Δ* and *ASE1-SF* cells show shorter and less dynamic spindles during HU treatment. *ase1Δ TUB1-GFP* cells containing a vector or *ASE1-SF* plasmid were treated and analyzed as described in Figure 4C. Spindle length over time is shown. **(C)** Induction of the expression of *ASE1-SF* from the *GAL* promoter. *ASE1-GFP TUB1-mCherry* cells containing a vector or P_{GAL}*-ASE1-SF-13myc* plasmid were grown to log phase in raffinose containing media. Galactose was added to final concentration of 2%. Cells were collected at the indicated time points and protein samples were prepared for western blotting using anti-myc antibody. Pgk1 protein levels are shown as a loading control. **(D)** Overexpression of *ASE1-SF* mimics *ase1Δ* phenotype. Saturated cell cultures with indicated genotypes containing P_{GAL}*-ASE1-SF* plasmid or vector were 10-fold serial diluted and spotted onto TRP dropout plates containing glucose or galactose and incubated at 30°C for 2 or 3 days before scanning. **(E)** Overexpression of *ASE1-SF* disrupts midzone localization of endogenous full-length Ase1 protein. *ASE1-GFP TUB1-mCherry* cells containing vector or P_{GAL}*-ASE1-SF* plasmid were grown to log phase in raffinose media. After release to galactose media for 1 hr, the cells were spotted onto the surface of a slide with an agarose galactose medium pad and subjected to live-cell microscopy at 25°C. Pictures from each time point for both GFP and mCherry were compiled to make videos. Images were selected and enhanced to show the bud morphology, spindle elongation and the localization of endogenous Ase1-GFP. Time zero was set to the first point of spindle elongation.

Table S1. Strains used in this study.

Strains	Relevant Genotypes	Reference
Y300	*MAT***a** *ura3-1 his3-11,15 leu2-3,112 trp1-1 ade2-1 can1-100*	Lab stock
972-5-3	*MAT***a** *ask1-3 ASE1-13myc::Sphis5*$^{+}$	This study
976-2-2	*MAT***a** *ASE1-13myc::Sphis5*$^{+}$	This study
973-1-1	*MAT***a** *ase1Δ::Sphis5*$^{+}$	This study
U960-5c	*MAT***a** *rad53Δ::HIS3 sml1-1*	Zhao X., et al.
2092-3-2	*MAT***a** *rad53Δ::HIS3 sml1-1 ASE1-13myc::Sphis5*$^{+}$	This study
2943-2-4	*MAT***a** *dun1Δ::Sphis5*$^{+}$ *ASE1-13myc::Sphis5*$^{+}$	This study
2219-2-3	*MAT***a** *ase1Δ::Sphis5*$^{+}$ *TUB1-GFP::URA3*	This study
2326-1-2	*MAT***a** *cdc13-1 ASE1-13myc::Sphis5*$^{+}$	This study
2903-15-1	*MAT***a** *ase1Δ::Sphis5*$^{+}$ *nip100Δ::KanMX*	This study
YHW305	*MAT***a** *cin8Δ::Sphis5*$^{+}$	Lab stock
YYW142-2	*MAT***a** *kar3Δ::Sphis5*$^{+}$	Lab stock
PSY1451	*MAT***a** *nip100Δ::Sphis5*$^{+}$	Lab stock
2567-2-1	*MAT***a** *ASE1-GFP::Sphis5*$^{+}$ *TUB1-mCherry::URA3*	This study
2991-3-1	*MAT***a** *ase1Δ::Sphis5*$^{+}$ *TUB1-mCherry::URA3*	This study
2023-10-1	*MAT***a** *cin8Δ::Sphis5*$^{+}$ *TUB1-GFP::URA3*	This study
2982-51-4	*MAT***a** *cin8Δ::Sphis5*$^{+}$ *ase1Δ::Sphis5*$^{+}$ *TUB1-GFP::URA3 + ase1-AA::TRP1*	This study
KM001	*MAT***a** *pre9Δ::KanMX*	ATCC

Experimental Procedures

Yeast Strains and Growth

The yeast strains used in this study are listed in Table S1. All strains are isogenic to Y300, a W303 derivative, except for *pre9Δ* mutants and the WT used in Figure S1C, which are from the ATCC deletion library. Yeast cells were grown in YPD (Yeast extract, Peptone, Dextrose) or indicated synthetic medium. To arrest cells in G_1 phase, 5 μg/ml α-factor was added into cell cultures. After 2 hr incubation, the G_1-arrested cells were washed with water then released into fresh medium to start the cell cycle. To induce *ASE1-SF* overexpression, cells were first grown in raffinose medium and galactose was added to the medium to a final concentration of 2%. Gene deletions and epitope tagging were performed by using a PCR-based protocol.

Plasmid Construction and Mutagenesis

All plasmids were constructed using a PCR-based method. PCR products were digested with restriction enzymes and then insertion into a *CEN-URA3, CEN-TRP1* or *CEN-TRP1-GAL* vector. For site directed mutagenesis, sets of 33-mer primers were used to change the 7^{th} and/or 8^{th} ATG to GCC on the plasmid containing the 13myc-tagged *ASE1* expressed from the endogenous promoter.

Cytological Techniques

Cells with GFP and mCherry-tagged proteins were fixed with 3.7% formaldehyde for 10 min at room temperature and then washed twice with 1xPBS buffer and resuspended in PBS buffer for fluorescence microscopy (Zeiss Axioplan2). Spindle morphology was monitored using *TUB1-GFP*. Live-cell microscopy was carried out with the Andor Revolution SD imaging system. We used a glass depression slide to prepare an agarose pad filled with synthetic complete

medium with the addition of galactose or 200 mM HU. All live-cell images were acquired at 25°C with a 100x objective lens. For *ase1-AA* and *ASE1-SF-GFP* experiments, a Z-stack with 13 planes separated by 0.5 μm was acquired at each time point and converted to maximum projection. Andor IQ2 software was used to manually measure the spindle length at each time point. For P_{GAL}*-ASE1-SF* experiments, a Z-stack with 15 planes separated by 0.3 μm was acquired at each time point and converted to maximum projection. Time-lapse interval was set at 3 min and maximum projection from applicable time points were created using Andor IQ2 software. Some images were selected and enhanced to show the bud morphology.

Protein Techniques

Cells pelleted from 1.5 ml of cell culture were resuspended in 200 μl 0.1 M NaOH and incubated at room temperature for 5 min. After centrifugation, the cells were resuspended in 100 μl 1xSDS loading buffer. The samples were boiled for 5 min and resolved with 8% SDS-polyacrylamide gel. Proteins were detected with ECL (Perkin Elmer LAS, Inc.) after probing with anti-myc or anti-HA antibodies (Covance Research Products, Inc.) and HRP-conjugated secondary antibody (Jackson ImmunoResearch, Inc).

RNA isolation, Northern Blotting and 5'-RACE

Strains used for northern blotting, 5'-RACE and RT-qPCR were treated as described in the corresponding figure legend, flash frozen with liquid nitrogen and stored at -80°C until RNA isolation. Total RNA was isolated using Qiagens RNEasy mini kit. Acid-washed glass beads were used to disrupt the cell wall by beating for 30 seconds with 1 min rest on ice 6 times. Genomic DNA was removed by incubating with TURBO DNase for 30 min at 37°C. RNA was cleaned and concentrated with Zymo RNA Clean and Concentrate-25 kit. Ambion NorthernMax kit and MAXIscript kit were used for standard northern analysis methods. 10 μg of RNA per

sample was run on a 1% formaldehyde-MOPS gel at 100 V for 3 hrs and transferred to nylon membrane, UV cross-linked and probed with ^{32}P-labeled *ASE1* RNA probe corresponding to nucleotides 2109 to 2411 of the *ASE1* gene. The probe was transcribed by addition of the T7 phage polymerase promoter. Membranes were then stripped and reprobed with ^{32}P-labeled probes corresponding to nucleotides 857 to 1170 of the *ACT1* gene. Membranes were exposed to a film with an intensifier for 24-72 hrs.

For 5'-RACE, an *ASE1* gene specific primer was used to generate cDNA. After the remaining RNA was removed, a poly-dC tail was added to the 3'-end of the cDNA. A primer that recognized the tail and a second gene specific primer were used to amplify the cDNA by PCR. The PCR products were then separated by gel electrophoresis, purified and ligated to TOPO vector for transformation to *E. coli*. Ten colonies from each PCR product were then sequenced. For RT-qPCR, random hexamers were used to generate cDNA (Invitrogen) from 4 μg of total RNA and real time PCR was performed using BioRad iQ5 thermocycler with primers from the indicated regions.