# Supporting Information

## Gowda et al. 10.1073/pnas.1216778110

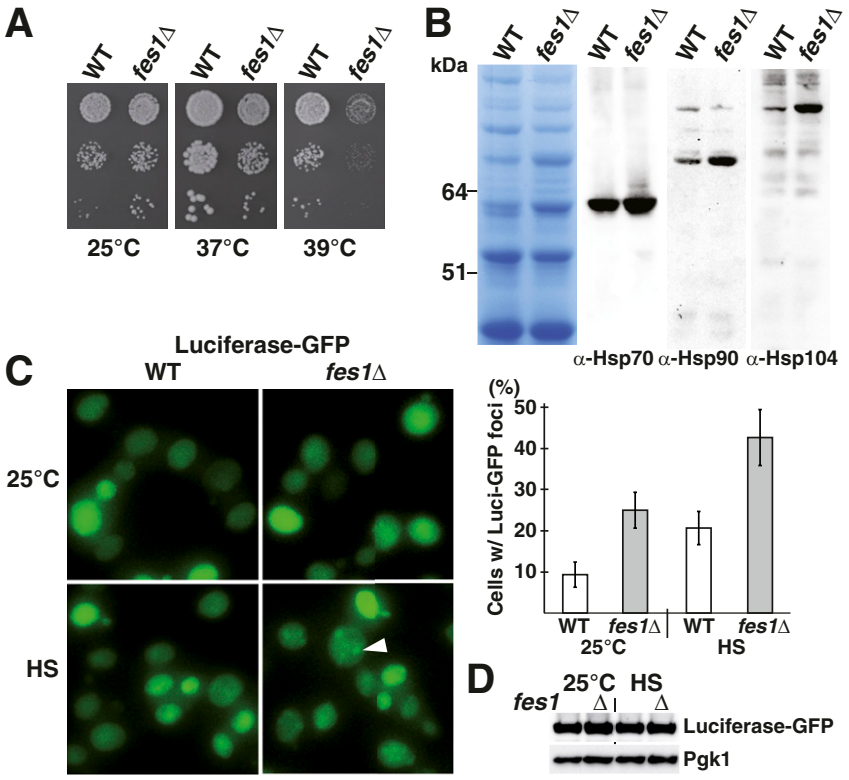


**Fig. S1.** *FES1* mutant cells are hypersensitive to conditions inducing protein misfolding conditions and accumulate protein aggregates. (*A*) *fes1*Δ strains are hypersensitive to elevated growth temperature. Tenfold dilutions of a series of cell suspensions were spotted onto YPD medium and were incubated at the indicated temperatures. (*B*) Total protein extracts from WT and *fes1*Δ cells grown in Yeast extract peptone dextrose (YPD) at 25 °C separated by SDS/PAGE and stained with Coomassie Brilliant Blue or analyzed by Western blotting with antibodies specific for Hsp70 (Ssa1/2 rabbit polyclonal serum), Hsp90 (monoclonal antibody K41220A), and Hsp104 (rabbit polyclonal serum). (*C*) Images of GFP fluorescence microcopy of fixed WT and *fes1*Δ cells expressing the aggregation reporter luciferase-GFP grown at 25 °C or heat shocked (HS) at 37 °C for 30 min. The arrow shows an aggregate. The aggregation data were quantified by counting the fraction of cells ($n = 30$) that contained detectable GFP aggregates. The error bars indicate the SD of three datasets from independent biological replicates. (*D*) Western analysis of luciferase (Luci)-GFP expression levels in the same strains under identical growth conditions as in *C*.

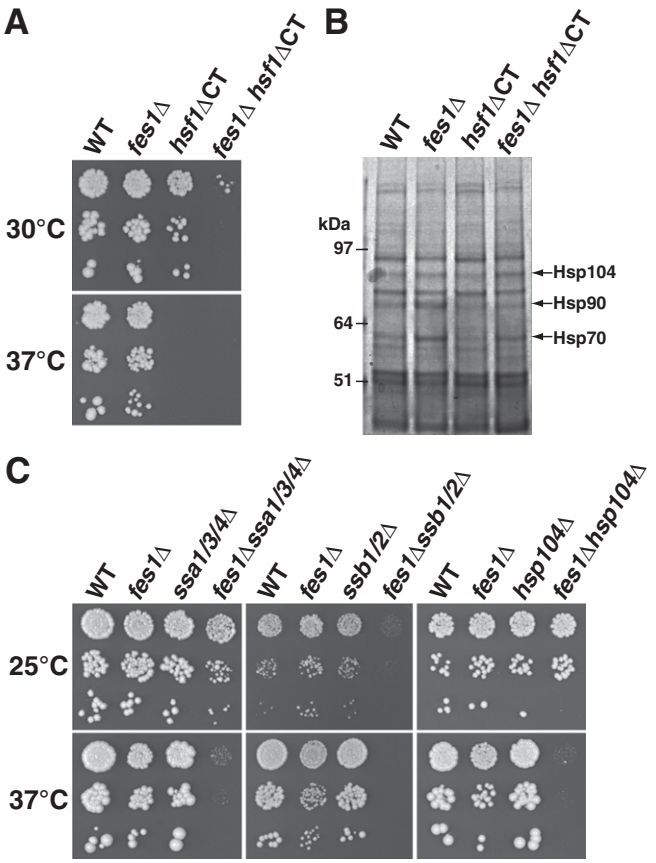


**Fig. S2.** Cells lacking *FES1* are hypersensitive to mutations that impair heat shock factor 1 (Hsf1) and chaperones Hsp70 and Hsp104. (*A*) Genetic interactions scored by growth phenotypes between *fes1*Δ and a mutation that impair Hsf1 activity from truncation of the C-terminal activation domain (*hsf1*ΔCT). (*B*) Heat shock induction (Hsp70, Hsp90, and Hsp104) in the indicated strains was assessed by SDS/PAGE. (*C*) Genetic interactions between *fes1*Δ and the genetic deletion of the three heat shock–inducible Hsp70s (*ssa1/3/4*Δ), of the ribosome-associated Hsp70s (*ssb1/2*Δ), or of the AAA+ ATPase and disaggregase Hsp104 (*hsp104*Δ).

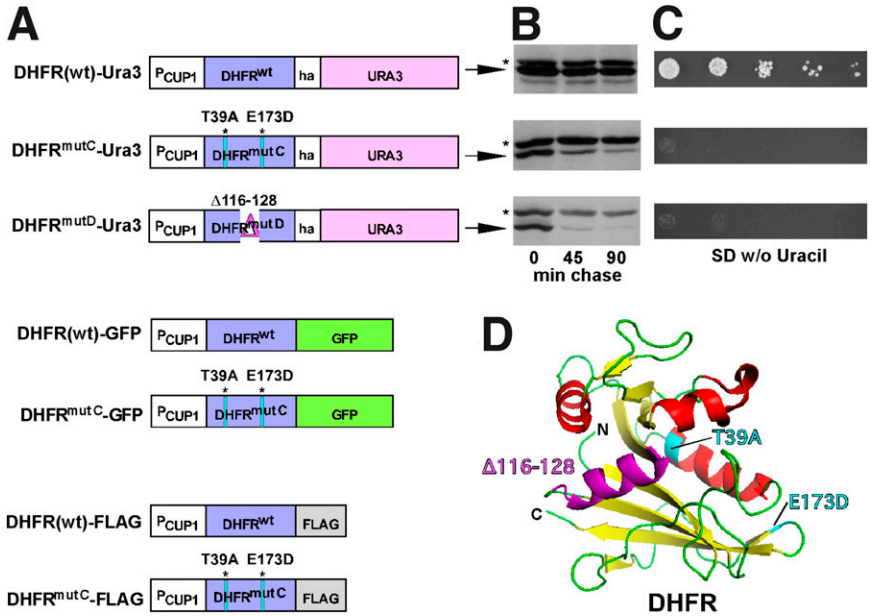


**Fig. S3.** Folding-deficient model substrates. (*A*) Shown are the DHFR constructs used in this study, all of which were expressed from the copper-inducible promoter ($P_{CUP}$) on centromeric plasmids and epitope tagged with human influenza hemagglutinin epitope (HA), the FLAG epitope (FLAG), or GFP. The Ura3 protein is the enzyme orotidine-5′-phosphate decarboxylate in uracil biosynthesis. (*B*) Cycloheximide chase assay of yeast cells expressing WT or the mutant DHFR-HA-Ura3 constructs. Proteins were detected by anti-HA Western blotting. A cross-reactive band is indicated by an asterisk. (*C*) Growth assay of yeast cells expressing the same constructs as in *B* on synthetic dextrose (SD) minimal media lacking uracil. (*D*) Illustration of the crystal structure of mouse Dihydrofolate reductase (DHFR). The α helix (residues 116–128) deleted in $DHFR^{mutD}$ is highlighted in purple. The two residues (T39A, E173D) that are mutated in $DHFR^{mutC}$ are highlighted in magenta.

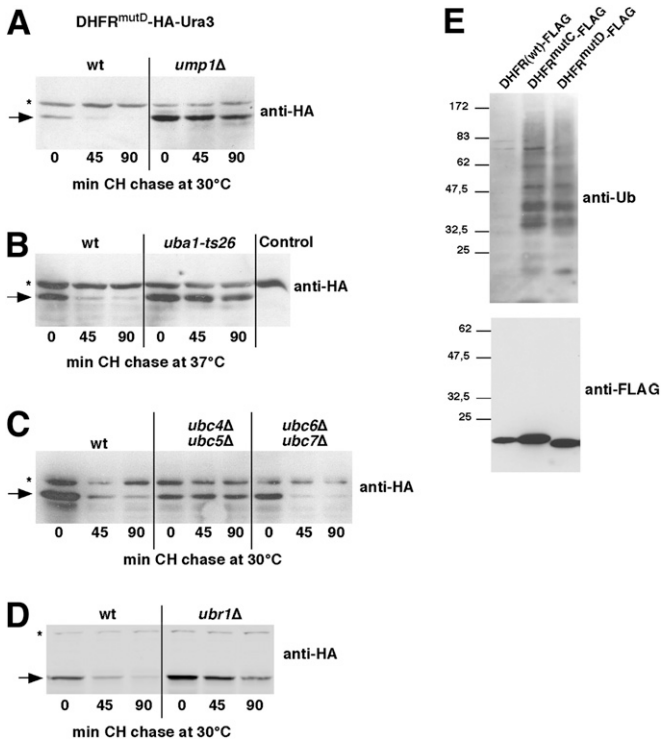


**Fig. S4.** Folding-deficient proteins are degraded by the ubiquitin/proteasome system (UPS). (*A*) Folding-deficient DHFR is stabilized in the proteasome-deficient *ump1*Δ mutant (1). Cycloheximide chase assay of DHFR$^{mutD}$-Ura3 stability in WT and *ump1*Δ strains. A cross-reactive band is indicated by an asterisk. (*B*) A functional ubiquitin-activating enzyme is required for the degradation of folding-deficient DHFR. Cycloheximide chase assay of DHFR$^{mutD}$-Ura3 in WT and *uba1-ts26,* a strain expressing a temperature-sensitive ubiquitin-activating enzyme (2). Cells were shifted to the restrictive temperature (37 °C) 20 min before the addition of cycloheximide. A cross-reactive band is indicated by an asterisk. (*C*) The redundant ubiquitin-conjugating enzymes Ubc4 or Ubc5 are required for degradation of folding-deficient DHFR (3). (*D*) DHFR$^{mutD}$-Ura3 degradation is attenuated in the *ubr1*Δ mutant lacking the ubiquitin ligase Ubr1 (4). (*E*) Folding-deficient DHFR is ubiquitylated in vivo. Protein extracts from WT yeast cells expressing FLAG-tagged versions of WT DHFR or its mutant derivatives were subjected to a pull-down experiment using anti-FLAG resin. Bound material was analyzed by anti-ubiquitin and, after stripping, by anti-FLAG Western blotting.


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2. Palanimurugan R, Scheel H, Hofmann K, Dohmen RJ (2004) Polyamines regulate their synthesis by inducing expression and blocking degradation of ODC antizyme. *EMBO J* 23(24):4857–4867.
3. Seufert W, Jentsch S (1990) Ubiquitin-conjugating enzymes UBC4 and UBC5 mediate selective degradation of short-lived and abnormal proteins. *EMBO J* 9(2):543–550.
4. Bartel B, Wünning I, Varshavsky A (1990) The recognition component of the N-end rule pathway. *EMBO J* 9(10):3179–3189.

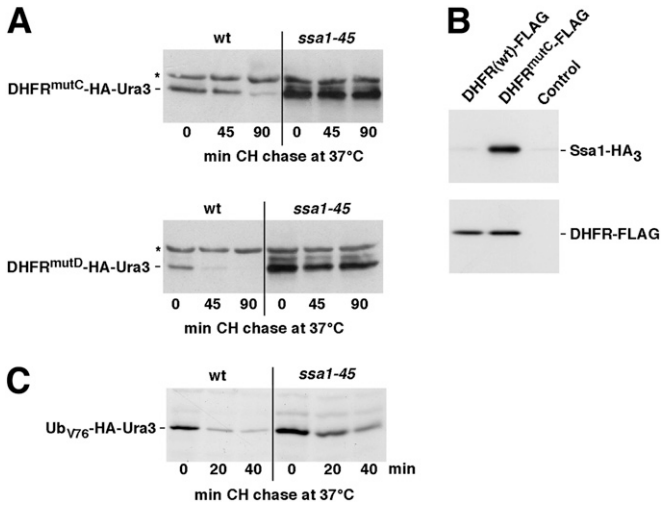


**Fig. S5.** Ssa class Hsp70 chaperones are required specifically for degradation of folding-deficient proteins. (*A*) Folding-deficient DHFR is stabilized in the *ssa1-45* mutant (1). Cycloheximide (CH) chase assay of DHFR$^{mutC}$-HA-Ura3 and DHFR$^{mutD}$-HA-Ura3 stability in WT and *ssa1-45* cells. Cells were shifted to the restrictive temperature (37 °C) 20 min before the addition of cycloheximide. (*B*) The Hsp70 chaperone Ssa1 is associated with folding-deficient DHFR. Protein extracts from a yeast strain expressing a 3xHA-tagged Ssa1 and FLAG-tagged versions of WT DHFR or its mutant derivative (mutC) were subjected to a pull-down experiment using anti-FLAG resin. Bound material was analyzed by anti-HA and, after stripping, by anti-FLAG Western blotting. The control strain expressed Ssa1-3xHA but no FLAG-tagged substrate. (*C*) Ssa class chaperones are not required for the degradation of the ubiquitin-fusion degradation (UFD) pathway substrate Ub$_{V76}$-HA-Ura3. Shown is a cycloheximide chase assay with the same strains as in *A* but with the UFD substrate. Cross-reactive bands are indicated by asterisks.

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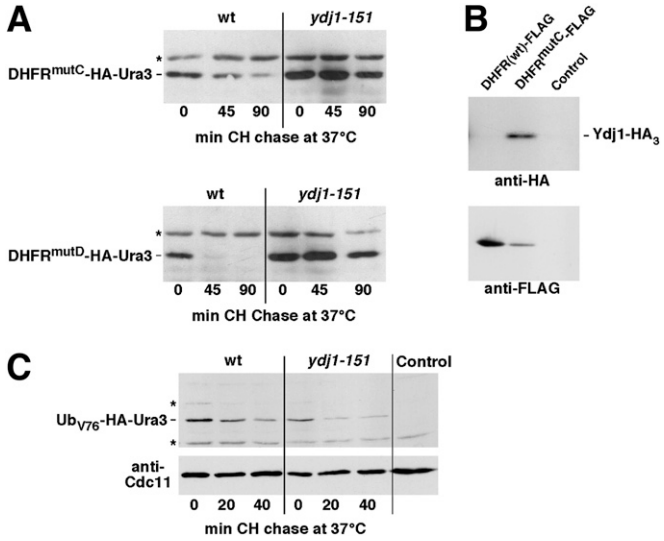


**Fig. S6.** Hsp40 Ydj1 is required specifically for degradation of folding-deficient proteins. (*A*) Folding-deficient DHFR is stabilized in the *ydj1-151* mutant. Cycloheximide chase assay of DHFR$^{mutC}$-HA-Ura3 and DHFR$^{mutD}$-HA-Ura3 stability in WT and *ydj1-151* cells (1, 2). Cells were shifted to the restrictive temperature (37 °C) 20 min before the addition of cycloheximide. A cross-reactive band is indicated by an asterisk. (*B*) The Hsp40 cochaperone Ydj1 is associated with folding-deficient DHFR. Protein extracts from a yeast strain expressing a 3xHA tagged Ydj1 and FLAG-tagged versions of WT DHFR or its mutant derivative (mutC) were subjected to a pull-down experiment using anti-FLAG resin. The control strain expressed Ydj1-3xHA but no FLAG-tagged substrate. Bound material was analyzed by anti-HA and, after stripping, by anti-FLAG Western blotting. (*C*) Cycloheximide chase assay showing degradation of the UFD pathway substrate Ub$_{V76}$-HA-Ura3 in WT and *ydj1-151* cells. Control, extract of WT cells with an empty vector. Cross-reactive bands are indicated by asterisks.

1. Caplan AJ, Cyr DM, Douglas MG (1992) YDJ1p facilitates polypeptide translocation across different intracellular membranes by a conserved mechanism. *Cell* 71(7):1143–1155.
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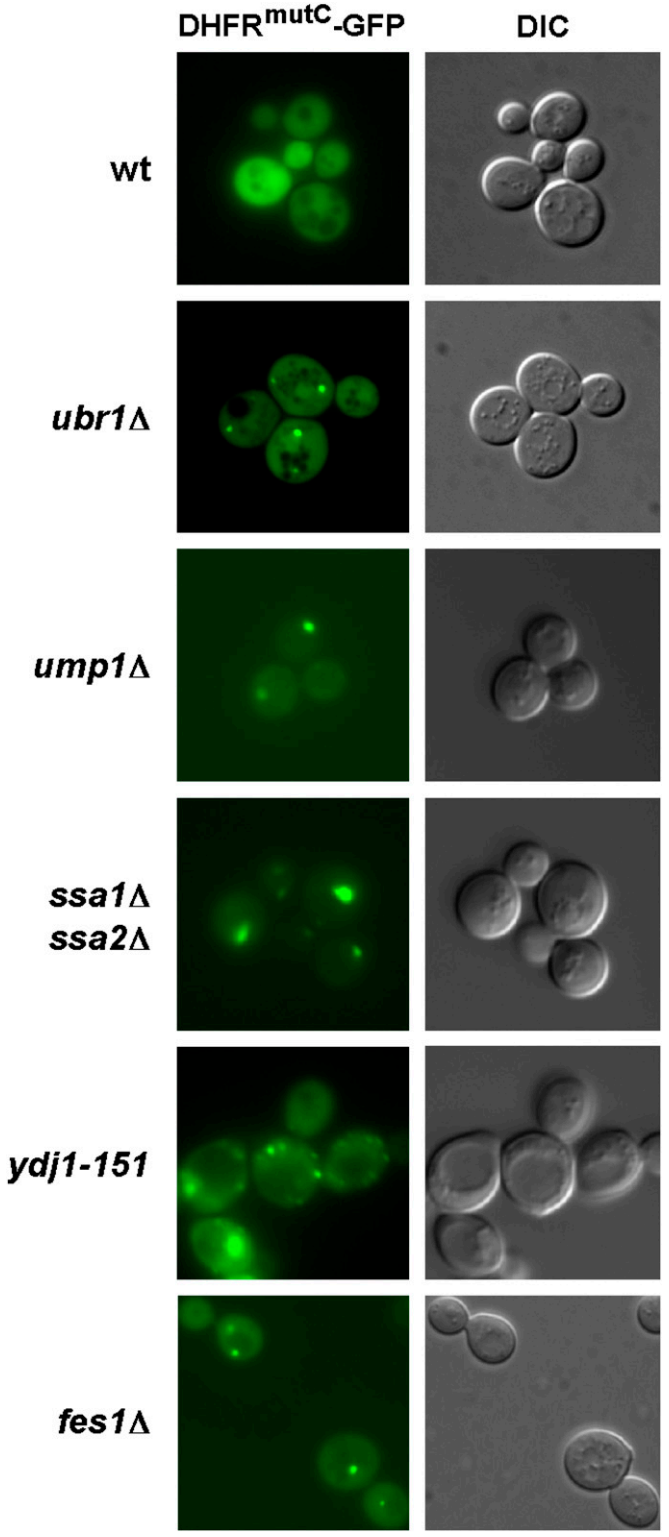


**Fig. S7.** Formation of intracellular aggregates of folding-deficient proteins. Cells with the indicated genotypes expressing DHFR$^{mutC}$-GFP were grown at 30 °C and shifted for 20 min to 37 °C before inspection in the fluorescence microscope (*Left*). (*Right*) Differential interference contrast (DIC) images of the same frames.

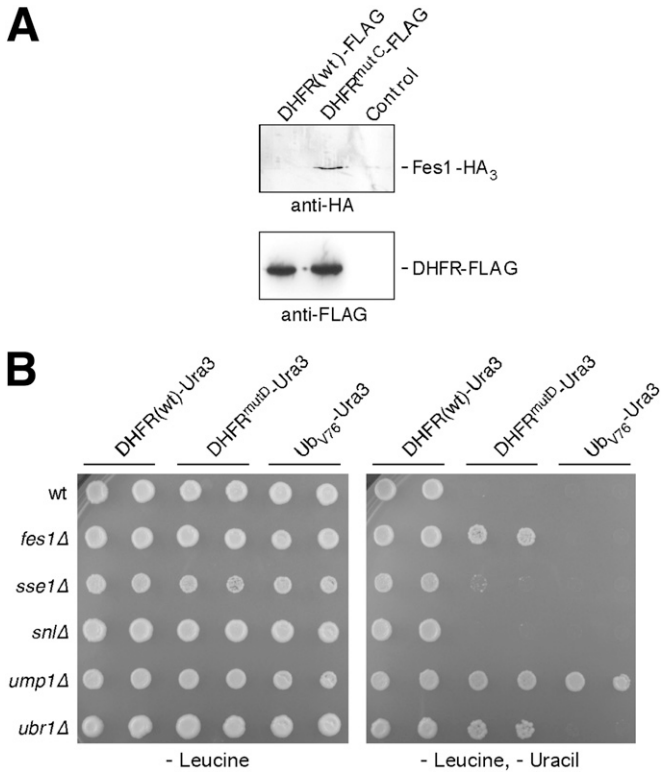


**Fig. S8.** Fes1 binds to misfolded proteins and promotes their degradation. (*A*) Fes1 is associated with folding-deficient DHFR. Protein extracts from a yeast strain expressing a 3xHA tagged Fes1 and FLAG-tagged versions of WT DHFR or its mutant derivative (mutC) were subjected to a pull-down experiment using anti-FLAG resin. The control strain expressed Fes1-3xHA but no FLAG-tagged substrate. Bound material was analyzed by anti-HA and, after stripping, by anti-FLAG Western blotting. (*B*) *Fes1*Δ cells are impaired in the degradation of folding-deficient $DHFR^{mutD}$-Ura3 protein. Two transformants each of WT, *fes1*Δ, *sse1*Δ, *snl1*Δ, *ump1*Δ, and *ubr1*Δ cells expressing the indicated proteins were grown to midlog phase and spotted at a concentration of 10 $OD_{600}$ per milliliter onto selective media lacking the indicated ingredients. Plates were incubated for 3 d at 30 °C.