Screen to identify new components in ERQD

Dieter H. Wolf

Institute of Biochemistry University of Stuttgart , Stuttgart, Germany

INTRODUCTION

For identification of new components involved in **e**ndoplasmic **r**eticulum **q**uality control and associated protein **d**egradation (ERQD), an available yeast mutant library is used for a genome-wide screen, which involves approximately 5000 mutant strains each deleted in a single nonessential gene (EUROSCARF, Frankfurt, Germany). Use of this genomic library is possible, because cells defective in ERQD can tolerate defects in this process as long as the **u**nfolded **p**rotein **r**esponse (UPR) is intact (Friedländer et al., 2000; Travers et al., 2000). A plasmid encoding the well characterized ERQD substrate CPY* fused to a transmembrane domain and the marker protein LEU2, called CTL*, is transformed into all 5000 mutants. In case of wild-type degradation of this chimeric protein, the cells are not able to grow on medium lacking the complementing nutrient. In contrast, disturbance of the ERQD pathway prevents the degradation of the ERQD substrate carrying the marker protein fusion and allows cell growth under selective conditions (Schäfer and Wolf, 2005).

MATERIALS

Standard yeast media and reagents are used **(**Sambrook et al., 1989)**.**

METHODS

1. Each deletion strain is precultured at 30°C overnight to stationary phase in sterile 24-well plates with lids containing 0.6 ml of YPD liquid medium in each well.
2. After inoculation of 60 µl of the preculture into new 24-well plates containing 0.6 ml YPD, the strains are regrown at 30°C for 2 h to logarithmic phase (A600 of 0.8–1.0/ml). The use of multichannel-pipettes makes the handling efficient.
3. After growth, cells are sedimented in the plates by centrifugation for 5–10 min.
4. The liquid supernatant is removed, and the sedimented cells are resuspended in sterile, deionized water followed by an additional centrifugation step.
5. Each strain is transformed with 2 µg of plasmid DNA that encodes the ERQD substrate protein CTL* together with 120 µg carrier DNA using the standard heat shock lithium acetate method (Gietz and Woods, 2002).
6. Add 600 µl of selection medium into each well, and cells are grown while shaking for 14–16 h at 30°C.
7. For selection of positive transformants containing the ERQD substrate encoding plasmid, 50 µl of each transformed strain is dropped on synthetic dropout medium plates without uracil (approximately 12 different strains per plate).
8. The plates are incubated for 2–3 days at 30°C.
9. For subsequent selection of mutants defective in ERQD, each growing strain is streaked on synthetic dropout medium without uracil and leucine (six mutants plus two control strains per plate).
10. As controls, ERQD wild-type and ERQD-defective strains (i.e., Δder3 ;Deak and Wolf, 2001; Gardner et al., 2000; Plemper et al., 1999) transformed with CTL* expressing plasmids were used.
11. Growth at 30°C will identify cells in which the degradation of the substrate CTL* is disturbed.
12. The growing yeast strains are retested by replating on dropout medium without uracil and leucine.

BIBLIOGRAPHY

Deak PM, Wolf DH. Membrane topology and function of Der3/Hrd1p as a ubiquitin-protein ligase (E3) involved in endoplasmic reticulum degradation. 2001. ***J Biol Chem.*** 276:10663-9

Gardner, R. G., Swarbrick, G. M., Bays, N. W., Cronin, S. R., Wilhovsky, S., Seelig, L., Kim, C., and Hampton, R. Y. (2000) ***J. Cell Biol.*** 151, 69-82

Gietz RD, Woods RA. Transformation of yeast by lithium acetate/single-stranded carrier DNA/polyethylene glycol method. 2002. ***Methods Enzymol.*** 350:87-96.

Friedlander R, Jarosch E, Urban J, Volkwein C, Sommer T. A regulatory link between ER-associated protein degradation and the unfolded-protein response. 2000. ***Nat Cell Biol.*** 2:379-84.

Plemper, R. K., and Wolf, D. H. Retrograde protein translocation: ERADication of secretory proteins in health and disease. 1999. ***Trends Biochem. Sci.*** 24, 266-270.

Sambrook, J., Maniatis, T., and Fritsch, E.F. (1989). ***Molecular cloning: a laboratory manual,*** 2nd edn (Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory Press).

Schäfer, A., and Wolf, D.H. (2005). Yeast genomics in the elucidation of endoplasmic reticulum (ER) quality control and associated protein degradation (ERQD). ***Methods in enzymology*** *399*, 459-468.

Travers KJ, Patil CK, Wodicka L, Lockhart DJ, Weissman JS, Walter P. Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. 2000. ***Cell.*** 101:249–258.