MOLECULAR AND CELLULAR BIOLOGY, Aug. 1992, p. 3663–3664
0270-7306/92/083663-02$02.00/0

Vol. 12, No. 8

Letter to the Editor
SEC22 and *SLY2* Are Identical

Genetic analysis of the secretory pathway in the yeast *Saccharomyces cerevisiae* has identified 11 *sec* and *bet* mutants that are temperature sensitive for transport from the endoplasmic reticulum (ER) to the Golgi complex (*sec12*, *-13*, *-16*, *-17*, *-18*, *-20*, *-21*, *-22*, and *-23* and *bet1* and *-2* [6, 8]). To clone *SEC22*, we transformed the *sec22-3* mutant with a library of yeast genomic DNA constructed in the multicopy vector YEP24 (2). We defined a 4.5-kb fragment that complemented this mutant at 37°C. Integration by homologous recombination was used to determine whether this DNA fragment contained the *SEC22* structural gene. This was done by ligating the 4.5-kb insert into YIp5, a *URA3*-containing plasmid that must be integrated into the yeast genome to be maintained (12). The resulting construct was digested within the insert to direct integration and then transformed into a *ura3 his4* strain. These events placed the *URA3* gene adjacent to the cloned locus. The Ura^+ strain was then crossed to a *ura3 sec22-3* mutant. The diploid was sporulated, and the Ts^+ and Ura^+ markers were found to cosegregate in 15 of 15 tetrads. This finding indicates that the cloned DNA contains the *SEC22* structural gene.

Further subcloning defined a 2.8-kb fragment and a 1.8-kb fragment (Fig. 1A and B) capable of full complementation. Digestion of the unique *Nco*I site followed by end filling and ligation resulted in a loss of complementing activity, defining this site as internal to the gene (Fig. 1E). DNA sequence analysis identified one open reading frame (of 0.64 kb) spanning this site, which is predicted to encode a 25-kDa protein. A comparison of this sequence with others in the National Biomedical Research Foundation Library data base revealed that *SEC22* is identical to *SLY2* (suppressor of loss of *YPT1* function). *SLY2* was identified as a gene whose overproduction enables yeast cells to survive in the absence of the essential *ras*-like Ypt1 protein (3, 4), which is required for ER-to-Golgi transport (1, 10, 11).

SEC22 interacts genetically with *BET1* and *BOS1*, two other genes required for ER-to-Golgi transport (7). To identify additional genetic interactions, one member of each complementation group of *sec* and *bet* mutants that block ER-to-Golgi transport was transformed with the *SEC22* gene on a multicopy plasmid. The *sec22-3* mutant defect was fully rescued, as expected, and *sec21-1* was suppressed (5). The *sec21-1* mutant grows at 30°C but not at 34°C. However, in the presence of the multicopy plasmid, the transformed *sec21* strain displays moderate growth at 34°C but is not rescued at 37°C. *SLY2* was found to suppress *sec21-1* over a similar temperature range (9). Only partial suppression of *sec22-3* by *SLY2* was reported (9). The reason for this is unclear, since a *Bam*HI-*Eco*RV fragment was used, which is presumably identical to the fully complementing plasmid pSFNB50 (Fig. 1D). However, the integration experiment and the DNA sequence identity unequivocally demonstrate that *SEC22* and *SLY2* are the same gene. The identity of *SEC22* and *SLY2* is further supported by the observation that antisera raised to either a *lacZ-SLY2* (9) or a *GST-SEC22* fusion protein recognize a band of 25 kDa that partitions with membranes.

We thank Melissa Clague and Caryn Hashimoto for technical assistance. We also thank Irene Griff for the comparison of *SEC22* with other sequences in the data base.

This work was supported by grants awarded to S.F-N from the National Institutes of Health (1 RO1 GM45431 and CA 46128).

REFERENCES

1. **Bacon, R. A., A. Salminen, H. Ruohola, P. Novick, and S. Ferro-Novick.** 1989. The GTP-binding protein, Ypt1, is required for transport *in vitro*: the Golgi apparatus is defective in *ypt1* mutants. J. Cell Biol. **109:**1015–1022.
2. **Carlson, M., and D. Botstein.** 1982. Two differentially regulated mRNAs with different 5′ ends encode secreted and intracellular forms of yeast invertase. Cell **28:**145–154.
3. **Dascher, C., R. Ossig, D. Gallwitz, and H. D. Schmitt.** 1991. Identification and structure of four yeast genes (*SLY*) that are able to suppress the functional loss of *YPT1*, a member of the *RAS* superfamily. Mol. Cell. Biol. **11:**872–885.
4. **Gallwitz, D., C. Donath, and C. Sander.** 1983. A yeast gene encoding a protein homologous to the human c-*has*/*bas* proto-oncogene product. Nature (London) **306:**704–707.
5. **Newman, A. P.** 1991. The role of the yeast *BET* and *BOS* genes in transport between the ER and the Golgi complex. Ph.D. thesis, Yale University, New Haven, Conn.
6. **Newman, A. P., and S. Ferro-Novick.** 1987. Characterization of new mutants in the early part of the yeast secretory pathway isolated by a ^{3}H-mannose suicide selection. J. Cell Biol. **105:** 1587–1594.
7. **Newman, A. P., J. Shim, and S. Ferro-Novick.** 1990. *BET1*, *BOS1*, and *SEC22* are members of a group of interacting yeast genes required for transport from the endoplasmic reticulum to the Golgi complex. Mol. Cell. Biol. **10:**3405–3414.
8. **Novick, P., C. Fields, and R. Schekman.** 1980. Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. Cell **21:**205–215.
9. **Ossig, R., C. Dascher, H.-H. Trepte, H. D. Schmitt, and D. Gallwitz.** 1991. The yeast *SLY* gene products, suppressors of defects in the essential GTP-binding Ypt1 protein, may act in endoplasmic reticulum-to-Golgi transport. Mol. Cell. Biol. **11:** 2980–2993.
10. **Schmitt, H. D., M. Puzicha, and D. Gallwitz.** 1988. Study of a temperature-sensitive mutant of the *ras*-related *YPT1* gene product in yeast suggests a role in the regulation of intracellular calcium. Cell **53:**635–647.
11. **Segev, N., J. Mulholland, and D. Botstein.** 1988. The yeast GTP-binding Ypt1 protein and a mammalian counterpart are associated with the secretion machinery. Cell **52:**915–924.
12. **Struhl, K., D. Stinchcomb, S. Scherer, and R. Davis.** 1979. High frequency transformation of yeast: autonomous replication of hybrid DNA molecules. Proc. Natl. Acad. Sci. USA **76:**1035–1039.

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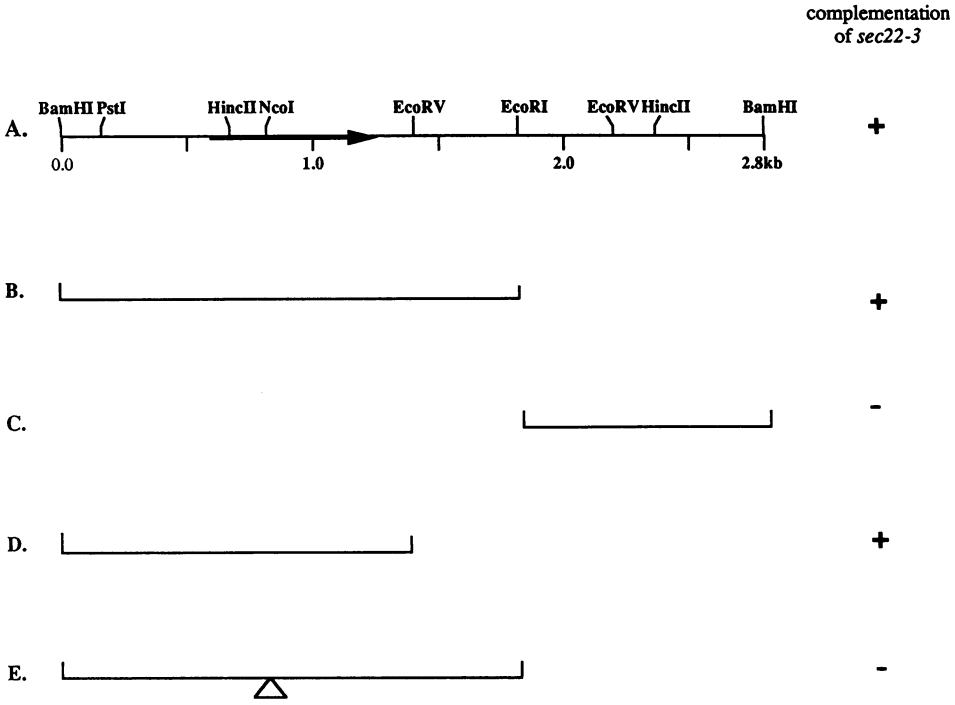

FIG. 1. Complementing activity of plasmids containing the *SEC22* gene. Only the cloned insert, and not the vector portion of each plasmid, is shown. (A) pJG103; (B) pSFNB30a; (C) pSFNB30b; (D) pSFNB50; (E) pSFNB82. pJG103 was constructed in the multicopy vector YEp24, and the others were constructed in the single-copy vector YCp50. The arrow in pJG103 indicates the direction and length of the *SEC22* coding sequence, and the triangle in pSFNB82 indicates the location of the frameshift mutation.

Author's Reply

In our previous reports on the cloning and functional analysis of yeast genes that are capable of suppressing *ypt1* loss-of-function mutants, we had shown that *SLY2*, *SLY12*, and *SLY1-20* could partially suppress a temperature-sensitive, secretion-defective *sec22* mutant (1, 2). After we were informed that *SLY2* and *SEC22* might be identical, we tested the genotype of the *sec22* mutant strain that we had received from another laboratory and had used in our earlier complementation studies (2). We discovered that this strain, and those derived from it, carried a Ts^- allele of *sec21* rather than *sec22*. We therefore reinvestigated the complementing activities of the different *SLY* genes (1, 2), using a *sec22-3* mutant provided by R. Schekman. In agreement with the results reported above by Newman et al., the cloned *SLY2* gene on a multicopy vector perfectly rescued the mutant growth defect at 37°C. In addition, diploid strains that were obtained by crossing the *sec22-3* mutant with a strain carrying the disrupted *SLY2* allele were still Ts^-, which also agrees with the above finding that *SLY2* and *SEC22* are in fact allelic.

In contrast to our previously published experiments in which we unknowingly used the wrong yeast strain, we now find that *SLY1-20*, a single-copy suppressor of *YPT1* disruptions (1, 2), as well as the multicopy suppressor *SLY12* (1, 2) allows the *sec22-3* mutant to grow at 36°C. However, *SLY41* (1, 2) did not improve the growth of this mutant at temperatures higher than 30°C. This shows that *SLY1-20* and *SLY12* are in fact more potent suppressors of *sec22* than of *sec21* mutants.

REFERENCES

1. **Dascher, C., R. Ossig, D. Gallwitz, and H. D. Schmitt.** 1991. Identification and structure of four yeast genes (*SLY*) that are able to suppress the functional loss of *YPT1*, a member of the *RAS* superfamily. Mol. Cell. Biol. **11**:872–885.
2. **Ossig, R., C. Dascher, H.-H. Trepte, H. D. Schmitt, and D. Gallwitz.** 1991. The yeast *SLY* gene products, suppressors of defects in the essential GTP-binding Ypt1 protein, may act in endoplasmic reticulum-to-Golgi transport. Mol. Cell. Biol. **11**: 2980–2993.

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