indirectly from the delay that begins before or at the time at which axial elements form, but we favor a different model.

Taken together, our results suggest that Ndj1p stabilizes homology-dependent interactions. These interactions may be similar in properties to the paranemic joins proposed by Weiner and Kleckner (*14*) and be telomere-specific, or, if Ndj1p also functions at nontelomeric sites, they might correspond to the same paranemic joins and simply be more abundant at the telomeres than interstitially. Given that *NDJ1* influences segregation of heterologs, which presumably are nonrecombinant, the proposed stabilization would occur independently of recombination. The concentration of this activity at the telomeres could account for the retention of association of telomere-adjacent regions even though interstitial interactions are lost in a recombination-defective mutant (*14*).

Although nondisjunction of homologs in *ndj1*Δ cells could result from relatively subtle defects in crossing-over (perhaps in specific regions or on specific chromosomes), the same phenotype would result from defective chiasma function as a result of decreased cohesion of sister chromatids (*15*). The increase in PSS and in meiosis II abnormalities in *ndj1*Δ cells could result from a failure of sister chromatid cohesion (*16*) specifically at the telomeres. In addition, Ndj1p could stabilize sister interactions interstitially, perhaps at hotspots of double-strand breakage, which have been demonstrated to reduce PSS (*9*). By holding sister chromatids together or initiating interactions, Ndj1p might facilitate axial element formation, although the precise mechanism for such an effect is unclear. Stabilization of interstitial homolog interactions could facilitate synapsis. Stabilization of telomere interactions would bring subtelomeric sequences into proximity (*17*) and hold chromosomes in register, which could also facilitate synapsis.

Ndj1p is required for linear heterologs to segregate distributively with the same efficiency as circular heterologs, which do not require this protein. Thus, in the absence of Ndj1p, telomeres interfere with the distributive segregation pathway.

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1. Nondisjunction was assayed in MCY387, a diploid strain in the SK1 strain background with the genotype *MAT***a**/*MATα leu2/leu2 his4::HIS3/HIS4 his3Δ200/his3Δ200 CAN1^s/can1^r CYH2^s/cyh2^r ade2-101/ADE2 lys2/lys2 ura3-52/ura3-52 trp1Δ1/trp1Δ1*. MCY387 is heterozygous for the recessive mutations *can1^r* and *cyh2^r*, which confer resistance to canavanine and cycloheximide, respectively. Thus one-quarter of all spores are resistant to both drugs. Spores that receive one copy of chromosome III are His$^-$. Disomic spores that receive *HIS4* and *his4::HIS3* are His$^+$. The rate of missegregation of chromosome III is given by the number of His$^+$ Can^r Cyh^r spores divided by the number of Can^r Cyh^r spores. MCY387 was transformed with a library of wild-type yeast genomic DNA fragments in the *URA3*, *leu2-d* vector pHR81 [J. O. Nehlin, M. Carlberg, H. Ronne, *Gene* **85**, 313 (1989)], and plasmid copy number was controlled by selection for *URA3* or *LEU2*. Plasmids that interfered with meiosis or sporulation were subjected to transposon mutagenesis [M. Strathman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 1247 (1991)] to map the region responsible for the meiotic effect. The *NDJ1* open reading frame was identified in three plasmids that caused increased nondisjunction with *URA3* selection (~30 copies per cell) and prevented sporulation with *LEU2* selection (~100 copies per cell).
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t-SNARE Activation Through Transient Interaction with a Rab-Like Guanosine Triphosphatase

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Intracellular vesicle targeting involves the interaction of vesicle proteins, termed v-SNAREs, with target membrane proteins, termed t-SNAREs. Assembly of v-SNARE–t-SNARE targeting complexes is modulated by members of the Sec1-Sly1 protein family, and by small guanosine triphosphatases termed Rabs. The interactions of these proteins during assembly of the endoplasmic reticulum–to-Golgi targeting complex in *Saccharomyces cerevisiae* were studied. The data suggest that the Rab protein Ypt1p transiently interacts with the t-SNARE Sed5p and results in displacement of the negative regulator Sly1p, allowing subsequent formation of the v-SNARE–t-SNARE targeting complex.

The movement of proteins between intracellular membrane-bounded compartments is mediated by transport vesicles that bud from one compartment and target specifically to the next compartment (*1*). Vesicle targeting involves interactions between integral membrane proteins, the v-SNAREs and t-SNAREs, that reside on vesicle and target membranes, respectively (*2*, *3*). The v/t-SNARE complex then binds soluble NSF attachment protein [SNAP, Sec17p in yeast (*4*)] and *N*-ethylmaleimide–sensitive fusion protein [NSF, Sec18p in yeast (*5*, *6*)], which catalyze the disassembly of the v/t-SNARE complex as a prelude to membrane fusion (*7*). Rab proteins, a family of small guanosine triphosphatases (GTPases) related to Ras, also act in vesicle targeting but their precise role is unclear (*8*).

Targeting of vesicles from the endoplasmic reticulum (ER) to the Golgi in yeast involves three v-SNAREs (Sec22p, Bet1p, and Bos1p) (*9*, *10*), the t-SNARE Sed5p (*11*), the Rab-like GTPase Ypt1p (*12*, *13*), and a peripheral membrane protein termed Sly1p (*14*) that associates with Sed5p (*10*). Although Sly1p is required for ER to Golgi transport (*15*, *16*), it may also act as a negative regulator (*17*).

To examine whether Ypt1p physically interacts with Sed5p, we immunoblotted Sed5p immunoprecipitates (IPs) from strains (*18*) with and without a high copy number (2 μm) *YPT1* plasmid (*19*) (Fig. 1A). A weak Ypt1p-Sed5p interaction was

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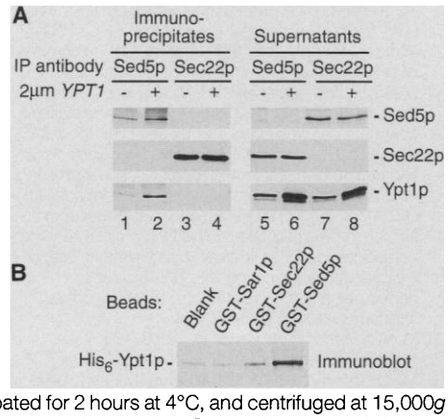

Fig. 1. Physical interaction of Ypt1p with Sed5p. (**A**) In vivo protein interaction. Spheroplasts of RSY255 (lanes 1, 3, 5, and 7) or RSY255 pNB167 (*YPT1*, *URA3*, 2 μm) (lanes 2, 4, 6, and 8) were lysed with nonionic detergent and proteins immunoprecipitated (IP) with antibody to Sed5p (anti-Sed5p) or anti-Sed22p (*28*). The IPs (lanes 1 to 4) and one-fifth of the corresponding supernatants (lanes 5 to 8) were then immunoblotted to detect Sed5p, Sec22p, and Ypt1p. (**B**) In vitro interaction. Ten micrograms of GST-Sar1p (~200 pmol), GST-Sec22p (~200 pmol), or GST-Sed5p (~150 pmol) in phosphate-buffered saline (PBS), or PBS alone (blank), was mixed with 0.2 μg of His_6-Ypt1p (~10 pmol) in 1 ml of buffer B [20 mM Hepes-KOH (pH 7.0), 150 mM potassium acetate, 0.5 mM dithiothreitol, 0.05% Tween-20], incubated for 2 hours at 4°C, and centrifuged at 15,000*g* for 15 min. The supernatant (0.95 ml) was incubated for 1 hour at 4°C with 40 μl of glutathione–Sepharose 4B equilibrated in buffer B. Beads were washed four times at 23°C with 1 ml of buffer B and transferred to another tube. Bound proteins were eluted with 20 μl of 10 mM reduced glutathione in 50 mM tris-HCl (pH 7.5), separated by SDS-PAGE, transferred onto nitrocellulose, stained with Ponceau S [which indicated that equivalent amounts of GST-fusion protein were recovered (*23*)], and immunoblotted with antibodies (*28*) to Ypt1p. About 8% of the His_6-Ypt1p present in the reaction was recovered with the GST-Sed5p beads.

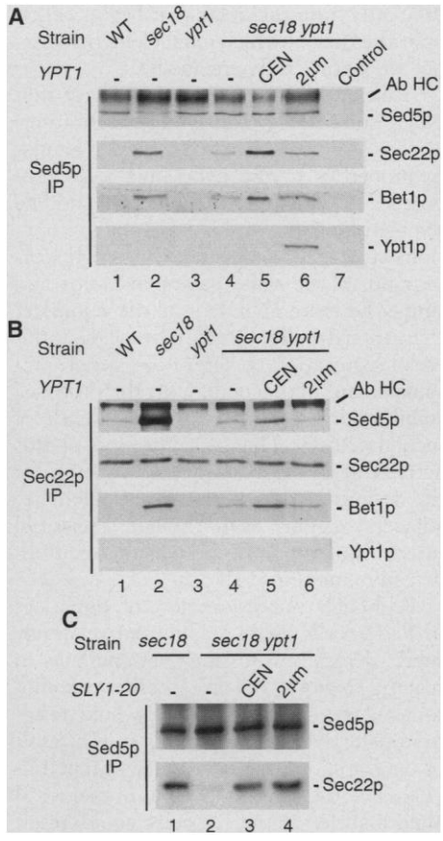

Fig. 2. Inhibition of v- and t-SNARE interaction after overexpression of Ypt1p. (**A**) Sed5p, Sec22p, Bet1p, and Ypt1p immunoblots of anti-Sed5p IPs from nonionic detergent–lysed spheroplasts shifted to 38°C for 20 min before lysis. (**B**) Same as (A) for anti-Sec22p IPs. Lane 1, RSY255 (WT); lane 2, RSY271 (*sec18-1*); lane 3, RSY976 (*ypt1-3*); lane 4 GWY141 (*sec18-1 ypt1-3*); lane 5, GWY141 pNB166 (*YPT1, URA3, CEN*); lane 6, GWY141 pNB167 (*YPT1, URA3*, 2 μm); lane 7, as in lane 6, but mock-immunoprecipitated without antibodies to Sed5p. The polypeptide evident above Sed5p is the heavy chain of the immunoprecipitating antibody (Ab HC). The method was as described (*29*) except that the spheroplasts were regenerated at 38°C. (**C**) Sed5p and Sec22p immunoblots of Sed5p IPs (*29*). Lane 1, RSY271 (*sec18-1*); lane 2, GWY141 (*sec18-1 ypt1-3*); lane 3, GWY141 pYCP50-*SLY1-20* (*SLY1-20, URA3*, CEN); lane 4, GWY141 pSLY1-20 (*SLY1-20, URA3*, 2 μm).

detected in wild-type cells, which was greatly enhanced by overexpression of Ypt1p. The Ypt1p-Sed5p interaction appeared to be specific because immunoprecipitation of the v-SNARE Sec22p from the same lysates did not yield detectable Ypt1p. All of the Sed5p or Sec22p was immunoprecipitated from the lysates, yet only a small fraction of the Ypt1p was recovered with the Sed5p. This suggests that the Ypt1p-Sed5p interaction is of low affinity or is transient.

To determine if the in vivo interaction of Sed5p with overexpressed Ypt1p was direct, we attempted to reconstitute this interaction with purified proteins (Fig. 1B). His_6-tagged Ypt1p (His_6-Ypt1p) was incubated with buffer, or with glutathione-S-transferase (GST)–Sar1p, GST-Sec22p, or GST-Sed5 fusion proteins, and the complexes were recovered on glutathione-Sepharose beads. Sar1p participates in the formation of ER-derived coat protein II (COP II)–coated vesicles (*20*), thus GST-Sar1p serves as a negative control. His_6-Ypt1p did not bind to GST-Sar1p, whereas a low but reproducible amount of His_6-Ypt1p was bound to GST-Sec22p. In contrast, His_6-Ypt1p bound efficiently to GST-Sed5p. This result corroborates the Sed5p-Ypt1p interaction observed in vivo and shows that the Sed5p-Ypt1p interaction is direct.

To explore whether the physical association of overexpressed Ypt1p with Sed5p influences the assembly of v/t-SNARE complexes in vivo, we immunoblotted Sed5p IPs from lysates of various mutant strains to determine if other components of the v/t-SNARE complex were present (Fig. 2A). As previously shown (*10*, *21*), the v-SNAREs Sec22p and Bet1p were not associated with Sed5p in wild-type cells, probably because of the transient nature of the v/t-SNARE complex. In contrast, Sec22p and Bet1p were associated with Sed5p in a *sec18* strain, which lacks NSF activity at the restrictive temperature (*6*) and therefore cannot disassemble the v/t-SNARE complex (*7*, *10*, *21*). The *ypt1* strain (*22*) at the restrictive temperature accumulated no v/t-SNARE complex. This result has been previously interpreted to suggest that Ypt1p is required for v/t-SNARE complex assembly (*10*). For a more rigorous test, we constructed a *ypt1 sec18* double-mutant strain. If Ypt1p is required for v/t-SNARE complex assembly, then the *ypt1* defect should block the *sec18*-dependent v/t-SNARE complex accumulation. We found this to be the case: Less Sec22p and Bet1p were associated with Sed5p in the *ypt1 sec18* double-mutant strain than in the *sec18* strain. Assembly of v/t-SNARE complexes in the *ypt1 sec18* strain was restored by *YPT1* on a low copy number centromere-based (CEN) plasmid, confirming that reduced assembly in the double mutant was due to the *ypt1* mutation. Complementation with the high copy number 2-μm *YPT1* plasmid was not as effective as that with the CEN plasmid and correlated with the association of Ypt1p with Sed5p. This Ypt1p-Sed5p interaction was apparently specific because it was dependent on immunoprecipitation with the Sed5p antibody; beads without antibody bound neither Sed5p nor Ypt1p from the same strain. These data confirm that Ypt1p is required for v/t-SNARE complex assembly in vivo (*10*) and suggest that large amounts of Ypt1p can interfere with the assembly of v/t-SNARE complexes by binding to Sed5p.

We also analyzed assembly of the v/t-SNARE complex by immunoprecipitation of Sec22p (Fig. 2B). The results indicated again that Ypt1p is required for v/t-SNARE complex assembly and that overexpression of Ypt1p inhibits the process. A small amount of overexpressed Ypt1p immunoprecipitated with Sec22p (Fig. 2B), but the amount was much less than that found in Sed5p IPs (Fig. 2A).

In all cases, Bet1p only associated with Sec22p under conditions of v/t-SNARE

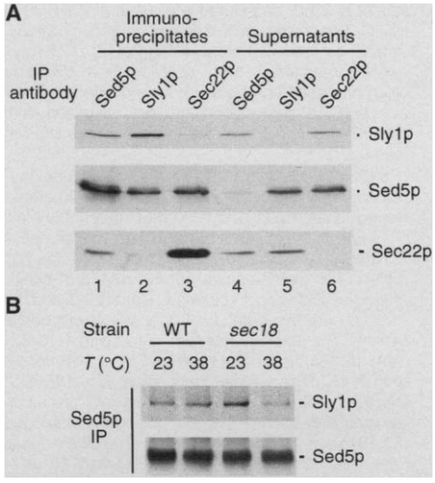

Fig. 3. Absence of Sly1p from v/t-SNARE complexes. (**A**) Sec22p, Sed5p, and Sly1p immunoblots of Sec22p, Sed5p, and Sly1p IPs (lanes 1 through 3) and one-fifth of the supernatants (lanes 4 to 6) from nonionic detergent-lysed RSY271 (*sec18-1*) spheroplasts. The method was as described (*29*) except that the spheroplasts were regenerated for 20 min at 38°C. (**B**) Sed5p and Sly1p immunoblots of denaturing Sed5p IPs from cross-linked lysates made from RSY255 (WT) and RSY271 (*sec18-1*) spheroplasts incubated at the permissive (23°C) and restrictive (38°C) temperature (*T*). Methods are described in (*30*).

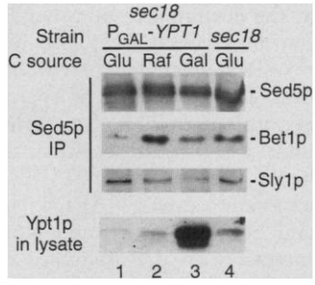

Fig. 4. Sensitivity of the Sed5p-Sly1p complex to the amount of Ypt1p. (Three upper panels) Sed5p, Bet1p, and Sly1p immunoblots of Sed5p IPs (*29*) from *sec18* cells incubated at the restrictive temperature and expressing various amounts of Ypt1p. (Bottom panel) Ypt1p immunoblot of the corresponding lysates. GWY142 (*sec18-1* P_{Gal}-YPT1) was grown to mid-logarithmic phase in YP media (*29*) containing 2% raffinose, pelleted, resuspended in fresh YP media containing either 4% glucose (Glu, lane 1), 2% raffinose (Raf, lane 2), or 2% galactose (Gal, lane 3), and grown for 4 hours at 23°C. RSY271 (*sec18-1*) was grown on YP and 4% glucose (Glu, lane 4). Spheroplasts were prepared and processed as described (*29*) except that they were regenerated for 60 min at 38°C in the same media in which they were grown supplemented with 0.8 M sorbitol. Controls without P_{Gal}-*YPT1* indicated that the various levels of Sed5p-Sly1p interaction were not a function of carbon source (*23*).

complex assembly (Fig. 2B), suggesting that the v-SNARE Bet1p only stably associates with the v-SNARE Sec22p in the v/t-SNARE complex. Association of the v-SNARE Bos1p with Sec22p was also enhanced in a *sec18* strain (*10*, *23*), suggesting that these v-SNAREs interact tightly with one another only in the v/t-SNARE complex.

The absence of Ypt1p activity in vivo leads to a growth defect and a block in ER to Golgi transport (*12*). These phenotypes can be suppressed by the presence of a dominant mutant allele of *SLY1*, termed *SLY1-20* (*14*, *15*). *SLY1-20* can also bypass the requirement for Ypt1p in v/t-SNARE complex assembly, because expression of Sly1-20p from a low copy number (CEN) plasmid in the *ypt1 sec18* double-mutant strain (Fig. 2C) almost completely restored v/t-SNARE complex assembly to the level obtained in the *sec18* strain; larger amounts of Sly1-20p expressed from a 2-μm plasmid completely restored v/t-SNARE complex assembly. The correlation of the *SLY1-20* genetics (*14*, *15*) with these results suggests that the immunoprecipitation technique faithfully measures v/t-SNARE complex assembly in vivo.

It has been proposed that Sly1p is a component of the yeast ER-Golgi v/t-SNARE complex (*10*). In contrast, the Sly1p homolog involved in synaptic vesicle docking, n-Sec1 (*24*), is not a v/t-SNARE complex component (*2*). We probed the association of a v-SNARE, the t-SNARE, and Sly1p with one another by immunoprecipitation under conditions that favor v/t-SNARE complex assembly, that is, in a *sec18* strain at the restrictive temperature (Fig. 3A). Both Sly1p and Sec22p were present in the Sed5p immunoprecipitate, but Sec22p was excluded from the Sly1p immunoprecipitate, and little Sly1p was in the Sec22p immunoprecipitate. This finding suggests that Sed5p is present in at least two different complexes: a Sed5p-Sly1p complex and a Sed5p-Sec22p complex, and that the v/t-SNARE complex does not contain Sly1p.

We also studied the Sed5p-Sly1p complex by cross-linking these proteins in yeast lysates (Fig. 3B). In lysates from wild-type cells, comparable amounts of Sly1p were cross-linked to Sed5p at 23° and 38°C. In a *sec18* strain, under conditions of v/t-SNARE complex accumulation (38°C), less Sly1p was cross-linked to Sed5p. Apparently, a large fraction of Sed5p was no longer associated with Sly1p, presumably because the Sed5p was present in v/t-SNARE complexes at the *sec18* restrictive temperature. Again, these results suggest that Sly1p is not a stable component of the yeast ER-Golgi v/t-SNARE complex, and that the interaction of Sly1p with Sed5p can be modulated.

The findings that Sly1p interacts with Sed5p before, but not after, formation of v/t-SNARE complexes, and that Ypt1p transiently interacts with Sed5p before assembly, prompted us to test whether Ypt1p influences the Sed5p-Sly1p interaction (Fig. 4). Different amounts of Ypt1p were expressed in a *sec18* strain with a regulatable *GAL10* promoter (*sec18* P_{Gal}-*YPT*), and proteins immunoprecipitating with Sed5p were examined by immunoblotting. A *sec18* strain was included as a control. Repression of Ypt1p synthesis in glucose-rich media precluded SNARE complex assembly, as indicated by the reduced association of the v-SNARE Bet1p with the t-SNARE Sed5p. Synthesis of approximately wild-type amounts of Ypt1p in raffinose media allowed SNARE complex assembly, whereas production of large amounts of Ypt1p in galactose-containing media less effectively promoted SNARE complex assembly. Examination of the amount of Sly1p associated with Sed5p at various Ypt1p levels showed that the Sed5p-Sly1p interaction was inversely proportional to the cellular amount of Ypt1p. This suggests that Ypt1p acts to disrupt or lower the affinity of the Sed5p-Sly1p interaction.

These results lead us to suggest a model for assembly of the v/t-SNARE vesicle-targeting complex that includes a specific role for the Rab-like GTPase Ypt1p (Fig. 5). We propose that before association of Sed5p with Sly1p, the t-SNARE is not competent for v-SNARE interaction (*25*). Association of Sly1p with Sed5p is required for subsequent t-SNARE functionality (*26*), but at the same time, Sly1p keeps the t-SNARE in an inactive state (*17*); this would explain both the positive and negative effects on transport of the Sly1p family. The displacement of Sly1p by Ypt1p, which may arrive

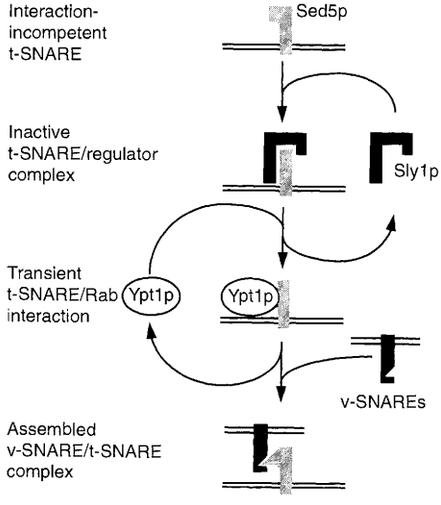

Fig. 5. Model for the regulation of v/t-SNARE complex assembly.

at the docking site in association with the transport vesicle (*13*, *27*), renders the t-SNARE competent for v-SNARE interaction. The interaction of Ypt1p with Sed5p is transient, and after, or perhaps concomitant with, its dissociation the activated Sed5p engages the v-SNAREs, resulting in an assembled v/t-SNARE complex. Sec17p and Sec18p (SNAP and NSF) can then interact with the assembled complex and catalyze disassembly (*7*), allowing membrane fusion to occur.

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18. *Saccharomyces cerevisiae* RSY255 (*MAT***a** *leu2-3-112 ura3-52*), RSY271 (*MAT***a** *sec18-1 his4-619 ura3-52*), RSY272 (*MAT***a** *sec18-1 his4-619 ura3-52*), and RSY976 (*MAT***a** *ypt1-3 ura3-52*) were from R. Schekman. GFUI-6D (*MAT***a** P_{Gal}-*YPT1 ΔYPT1::HIS3 ura3-52 leu2-3-112 trp1 his3*) was from H. D. Schmitt. GWY141 (*MAT***a** *sec18-1 ypt1-3 ura3-52*) was obtained from a cross of RSY976 and RSY272 strains. GWY142 (*MAT***a** *sec18-1* P_{Gal}-*YPT1 ΔYPT1::HIS his 4-619 ura3-52*) was obtained from a cross of RSY272 and GFUI-6D. Genotypes were confirmed by complementation analysis. Genetic manipulations were performed as described [M. Rose, F. Winston, P. Hieter, *Methods in Yeast Genetics: A Laboratory Course Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1990)].
19. The plasmids used were as follows: pQE9-Ypt1 [full-length Bam HI–Eco RI *YPT1* polymerase chain reaction (PCR) product was inserted into pQE9 (QIAGEN)], pGST-Sed5 [Bam HI–Eco RI PCR product encoding the cytoplasmic domain of Sed5p was inserted into pGEX2T (Pharmacia)], pGEXSar1 (full-length GST-Sar1p fusion from R. Schekman), pGEX-Sec22 [Bam HI–Eco RI PCR product encoding the cytoplasmic domain of Sec22 was inserted into pGEX2T (Pharmacia) from R. Schekman]. pNB166 (*YPT1 URA3, CEN*) and pNB167 (*YPT1 URA3*, 2 μm) were from S. Ferro-Novick. pYCP50-*SLY1-20* (*SLY1-20, URA3, CEN*) was from H. D. Schmitt. pSLY1-20 (*SLY1-20, URA3*, 2 μm) was constructed [Acc I–Cla I fragment of *SLY1-20* inserted into pRS426 [R. S. Sikorski and P. Hieter, *Genetics* **122**, 19 (1989)]] in our laboratory by S. K. Sapperstein.
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22. The *ypt1-3* allele was sequenced and had a C-to-A nucleotide change at position 284 of *YPT1* (GenBank number D50617, open reading frame YFL038C), which results in a serine-to-tyrosine change at residue 95 of Ypt1p.
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28. Rabbit polyclonal antibodies were raised to recombinant Sed5p-His_6, GST-Sec22p, GST-Sly1p, or GST-Ypt1p and affinity-purified by standard techniques [E. Harlow and D. Lane, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988)]. Antibodies to Bet1p were from R. Schekman.
29. Cells were grown to mid-logarithmic phase at 23°C in either synthetic complete (SC) or SC-Ura media supplemented with 1% casamino acids, harvested by centrifugation (5000*g*, 5 min), converted to spheroplasts (*10*), regenerated during a 20-min incubation in YP media (1% yeast extract, 2% peptone) with 1% glucose and 0.8 M sorbitol at 23°C, and lysed (*10*). The lysate was centrifuged at 15,000*g* for 15 min at 4°C. A 0.5-mg amount of supernatant protein (usually about 100 μl) was diluted to 1 ml in IP buffer [150 mM NaCl, 1% Triton X-100, 15 mM tris-HCl, (pH 7.5)] and mixed gently for 14 hours at 4°C with 15 μl of protein A–Sepharose beads bearing covalently attached affinity-purified antibodies (*28*) as indicated in the figure legends. The beads were washed four times with 1 ml of IP buffer at 23°C, and eluted with 40 μl of 2% SDS at 90°C. The eluates were resolved by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) (12% gel), electro-transferred onto polyvinylidene difluoride membrane, and probed with the indicated antibodies (*28*). Bound antibodies were visualized with chemiluminescent detection (Renaissance, DuPont NEN).
30. RSY255 and RSY271 spheroplasts (*29*) were incubated for 20 min at 23° or 38°C, sedimented, transferred to ice, and lysed in 20 mM Hepes-KOH (pH 7.0) and 150 mM potassium acetate with protease inhibitors (*10*) by gentle pipetting for 10 min. One-half volume of freshly prepared 10 mM 3,3′-dithiobis(sulfosuccinimidylpropionate) was added, and the reaction was incubated for 1 hour at 4°C with gentle mixing. The reaction was terminated with 1/10 volume of 1 M tris-HCl (pH 7.4) and incubation for 10 min at 4°C. The samples were adjusted to 1% SDS, heated to 90°C for 5 min, and diluted with nine volumes of IP buffer (*29*). The samples were centrifuged for 10 min at 15,000*g*, and 0.5 mg of soluble material was incubated for 4 hours at 23°C with 15 μl of protein A–Sepharose beads bearing covalently attached antibodies (*28*) to Sed5p. The samples were washed four times with IP buffer and eluted with 40 μl of 2% SDS at 90°C. The eluates were separated from the beads, 10 μl of 0.5 M dithiothreitol was added, and the samples were incubated for 20 min at 23°C. Samples (15 μl) were separated by SDS-PAGE (12% gel) and immunoblotted with antibodies (*28*) to Sed5p or Sly1p.

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Promoter Recognition As Measured by Binding of Polymerase to Nontemplate Strand Oligonucleotide

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In transcription initiation, the DNA strands must be separated to expose the template to RNA polymerase. As the closed initiation complex is converted to an open one, specific protein-DNA interactions involving bases of the nontemplate strand form and stabilize the promoter complex in the region of unwinding. Specific interaction between RNA polymerase and the promoter in *Escherichia coli* was detected and quantified as the binding affinity of nontemplate oligonucleotide sequences. The RNA polymerase subunit sigma factor 70 contacted the bases of the nontemplate DNA strand through its conserved region 2; a mutation that affected promoter function altered the binding affinity of the oligonucleotide to the enzyme.

Escherichia coli RNA polymerase exists in two forms (*1*): a core enzyme (E) that consists of subunits $\beta\beta'\alpha_2$ and is sufficient for elongation, and a holoenzyme ($E\sigma^{70}$) that includes E and a sigma polypeptide required for specific initiation of transcription—usually the sigma factor 70 (σ^{70}). The holoenzyme recognizes primarily two hexameric sequences centered at −10 and −33, with +1 being the start of transcription; the −10 element is included in the region of initial promoter opening. The specificity for promoter recognition is carried by σ^{70}, which can bind to double-stranded promoter DNA (*2*). It is likely, however, that $E\sigma^{70}$ mediates promoter opening.

$E\sigma^{70}$-DNA interactions that form and stabilize the open-promoter complex in the region of unwinding involve primarily

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