ation and editing activities. The anticodon stem-loop of $tRNA^{Ile}$ was introduced into $tRNA^{Val/GAU}$. Although this construct, like $tRNA^{Val/GAU}$, has an aminoacylation activity within about twofold that of native $tRNA^{Ile}$, it was not active in triggering the editing reaction (Fig. 2A). Similarly, introduction of the acceptor stem of $tRNA^{Ile}$ into $tRNA^{Val/GAU}$ yielded a molecule active for aminoacylation but not for editing.

The acceptor stem of $tRNA^{Val}$ was combined with the remaining three-quarters of $tRNA^{Ile}$, and the resulting molecule was almost fully active in both aminoacylation and editing (Fig. 2B). Thus, the origin of the acceptor stem, whether $tRNA^{Ile}$ or $tRNA^{Val}$, does not affect editing. Instead, the nucleotides required for editing reside in part or all of the D-loop (the sequences of the D-stem of $tRNA^{Ile}$ and $tRNA^{Val}$ are identical) or the TψC-stem-loop of $tRNA^{Ile}$. To test this hypothesis, we installed the D-loop from $tRNA^{Ile}$ into $tRNA^{Val/GAU}$ by changing three nucleotides (including an inserted nucleotide) of $tRNA^{Val/GAU}$. This construct was nearly fully active in aminoacylation and editing (Fig. 2B). Thus, a D-loop swap was sufficient to trigger the editing response but had little or no effect on the aminoacylation function. Next, we introduced the D-loop of $tRNA^{Val}$ into native $tRNA^{Ile}$. This molecule was almost fully active in aminoacylation but was inactive for editing (Fig. 2B). Therefore, regardless of whether the core framework is derived from $tRNA^{Ile}$ or $tRNA^{Val/GAU}$, the sequence of the D-loop is sufficient to trigger the editing response.

The D-loop interacts with the TψC-loop to form the corner of the two-domain L-shaped tRNA molecule (*15–17*) (Fig. 3). In this location the D-loop is positioned to be sensitive to simultaneous synthetase interactions with the anticodon and with the amino acid attachment site. This positioning may function in matching an amino acid with its corresponding anticodon. The editing system displays a high degree of specificity for a particular D-loop sequence which, in turn, might mediate a subtle conformational change that is necessary for triggering the editing response. This corner of the tRNA molecule is highly differentiated and might play a role in a protein-discrimination reaction (*17*). One possibility is that the large insertion into the active site of IleRS (referred to as CP1) interacts with this corner of the tRNA structure. This insertion is known to function in editing (*10*, *18*).

REFERENCES AND NOTES

1. T. Muramatsu *et al.*, *Nature* **336**, 179 (1988).
2. L. H. Schulman, *Prog. Nucleic Acid Res. Mol. Biol.* **41**, 23 (1991); R. Giegé, J. D. Puglisi, C. Florentz, *ibid.* **45**, 129 (1993); M. E. Saks, J. R. Sampson, J. N. Abelson, *Science* **263**, 191 (1994).
3. A. N. Baldwin and P. Berg, *J. Biol. Chem.* **241**, 839 (1966).
4. L. Pauling, in *Festschrift fuer Prof. Dr. Arthur Stoll Siebzigsten* (Birkhayser-Verlag, Basel, Switzerland, 1958), pp. 597–602.
5. E. Schmidt and P. Schimmel, *Science* **264**, 265 (1994).
6. H. Jakubowski and E. Goldman, *Microbiol. Rev.* **56**, 412 (1992); R. B. Loftfield, *Biochem. J.* **89**, 82 (1963).
7. A. R. Fersht and C. Dingwall, *Biochemistry* **18**, 2627 (1979).
8. A. Fersht, in *Enzyme Structure and Mechanism* (Freeman, New York, ed. 2, 1985), pp. 347–368.
9. E. W. Eldred and P. Schimmel, *J. Biol. Chem.* **247**, 2961 (1972).
10. L. Lin, S. P. Hale, P. Schimmel, *Nature* **384**, 33 (1996).
11. E. Schmidt, thesis, Massachusetts Institute of Technology, Cambridge, MA (1996), pp. 120–122.
12. S. P. Hale, D. S. Auld, E. Schmidt, P. Schimmel, data not shown.
13. The tRNA genes were constructed by extension of complementary oligonucleotides with Sequenase (U.S. Biochemical Corp., Cleveland, OH) followed by restriction and ligation into the Eco RI and Bam HI cloning sites of the phagemid pDSH100 (*19*). Phagemid pDSH100 allows for the in vivo overexpression of tRNA genes from a *lac*-inducible *tac* promoter. The *Escherichia coli* strain TG1 was used as a host strain for the in vivo expression of tRNAs. Cultures induced by isopropyl-β-D-thiogalactopyranoside were subjected to acid-buffered phenol extraction (*20*), followed by isolation of total tRNA by anion-exchange chromatography on Nucleobond AX-500 cartridges (Macherey-Nagel, Duren, Germany). Fractionation of tRNA was accomplished by reversed-phase high-performance liquid chromatography with a C4 column (Vydac, Hesperia, CA). Total tRNA samples were loaded onto the C4 column in 50 mM 2-[morpholino]-ethane sulfonate (pH 6.5), 1 M NH_4SO_4, 0.75% isopropanol, and eluted with a linear gradient of 50 mM 2-[morpholino]-ethane sulfonate (pH 6.5), 0.75% isopropanol. Pooled fractions were exchanged into 10 mM tris-HCl (pH 7.5), 1 mM EDTA and concentrated with Centricon-10 concentrators (Amicon, Beverly, MA). Purity of $tRNA^{Ile}$ was determined by polyacrylamide gel electrophoresis, Northern (RNA) blot analysis (*19*), and isoleucine acceptance (*21*). A 25 cm by 16 cm (1 mm thick) gel was cast with 12% acrylamide (29:1 acrylamide:bis-acrylamide in tris-borate EDTA buffer containing 8 M urea). The appropriate tRNAs (~3 μg) were loaded onto the gel and subjected to electrophoresis at 180 V for 18 to 20 hours. The gel was blotted with positively charged Qiabrane nylon membrane (Qiagen, Germany) by placing it in a wet Whatman filter-paper sandwich and dried at 80°C for 2 hours. The gel was then washed in 5× Denhardt's reagent–5× SSC [75 mM sodium citrate (pH 7.0), 750 mM sodium choloride] 0.5% SDS for 2 hours at 37°C. Then, 5 to 20 pmol of ^{32}P-labeled oligonucleotide complementary to either wild-type $tRNA^{Ile}$ or to a particular chimeric tRNA was added, and hybridization was allowed to proceed overnight at 37°C. The blot was then washed with 2× SSC–0.1% SDS for 15 min followed by 1× SSC–0.1% SDS for 15 min at ambient temperature. The bands were visualized with a Molecular Dynamics PhosphorImager.
14. O. Nureki *et al.*, *J. Mol. Biol.* **236**, 710 (1994).
15. J. D. Robertus *et al.*, *Nature* **250**, 546 (1974).
16. S. H. Kim *et al.*, *Science* **185**, 435 (1974).
17. J. E. Ladner *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4414 (1975).
18. E. Schmidt and P. Schimmel, *Biochemistry* **34**, 11204 (1995).
19. D. Hipps and P. Schimmel, *EMBO J.* **14**, 4050 (1995).
20. U. Varshney, C.-P. Lee, U. L. RajBhandary, *J. Biol. Chem.* **266**, 24712 (1991).
21. *Escherichia coli* isoleucine tRNA synthetase was isolated and aminoacylation assays were done as described (*22*). Valine-dependent tRNA-induced editing assays were done as described (*23*).
22. A. Shepard, K. Shiba, P. Schimmel, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 9964 (1992).
23. S. P. Hale and P. Schimmel, *ibid.* **93**, 2755 (1996).

24. Supported by grant GM15539 from the NIH. S.P.H. is an NIH postdoctoral fellow.

21 January 1997; accepted 7 April 1997

Ndj1p, a Meiotic Telomere Protein Required for Normal Chromosome Synapsis and Segregation in Yeast

Michael N. Conrad, Ana M. Dominguez, Michael E. Dresser*

The *Saccharomyces cerevisiae* gene *NDJ1* (nondisjunction) encodes a protein that accumulates at telomeres during meiotic prophase. Deletion of *NDJ1* (*ndj1*Δ) caused nondisjunction, impaired distributive segregation of linear chromosomes, and disordered the distribution of telomeric Rap1p, but it did not affect distributive segregation of circular plasmids. Induction of meiotic recombination and the extent of crossing-over were largely normal in *ndj1*Δ cells, but formation of axial elements and synapsis were delayed. Thus, Ndj1p may stabilize homologous DNA interactions at telomeres, and possibly at other sites, and it is required for a telomere activity in distributive segregation.

Efficient segregation of chromosomes in meiosis is required for haploidization. In a screen for yeast genes that control meiotic chromosome segregation, we identified *NDJ1* by its ability to interfere with this process when overexpressed (*1*). *NDJ1* corresponds to the open reading frame *YOL104c* from chromosome XV (*2*). The predicted Ndj1 protein comprises 352 amino acids and shows no significant similarities to other proteins. *NDJ1* was previously identified as the sporulation-specific gene M45 (*3*). A 1.4-kb *NDJ1* mRNA was detected by Northern blot analysis in yeast cells early in meiosis, but it was not detect-

Program in Molecular and Cell Biology, Oklahoma Medical Research Foundation, 825 Northeast 13th Street, Oklahoma City, OK 73104, USA.

*To whom correspondence should be addressed. E-mail: dresserm@omrf.uokhsc.edu

ed in vegetative cells (*4*).

Deletion of *NDJ1* (*ndj1*Δ) reduced sporulation efficiency (73 versus 88%), increased the number of asci with one or two versus three or four spores (15 versus 7%), and reduced spore viability (Table 1) (*3*), as compared with the wild type. Furthermore, in *ndj1*Δ homozygotes, the frequency of spores disomic for chromosome III increased 25-fold from wild-type values of $(7.0 \pm 0.6) \times 10^{-4}$, consistent with previous measurements (*5*), to $(1.8 \pm 0.2) \times 10^{-2}$. Thus, *NDJ1* is required for efficient segregation of chromosomes in meiosis.

Efficient orientation of chromosomes at meiosis I generally requires crossing-over and chiasma formation (*6*, *7*). However, there was no consistent difference between wild-type and *ndj1*Δ cells in the final extent of crossing-over or crossover interference (Table 2), or in the kinetics and yields of gene conversion events (Fig. 1A). Thus, recombination pathways are largely intact in *ndj1*Δ cells.

To determine the type of segregation errors in *ndj1*Δ cells, we studied the segregation of homologous or heterologous pairs of yeast artificial chromosomes (YACs) (*8*). In wild-type cells, the homologous YAC pair undergoes recombination and disjunction at a frequency similar to that for genuine yeast chromosomes, whereas the heterologous YAC pair does not recombine but nevertheless undergoes disjunction at meiosis I (Table 3) (*8*, *9*) by distributive segregation, which in yeast is less efficient than chiasmate segregation (*8*, *10*). Disjunction of heterologous YACs was markedly reduced in *ndj1*Δ cells (Table 3) (random segregation would be 50%), establishing that *NDJ1* is required for their distributive segregation. Increases in precocious sister separation (PSS) and double precocious sister separation (PSS×2) as well as in meiosis

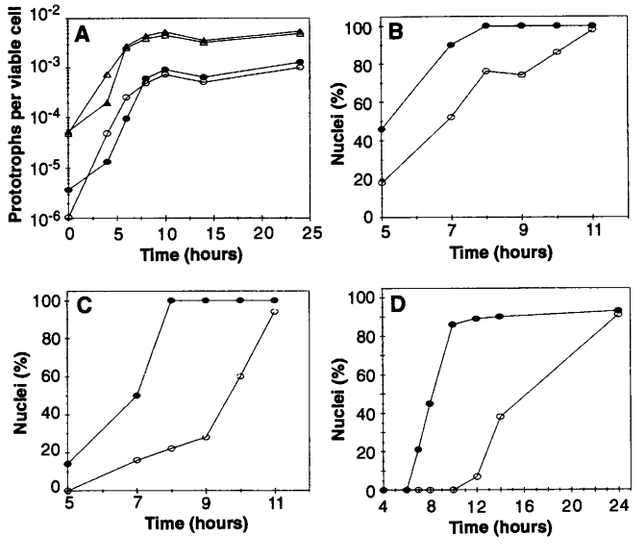

Fig. 1. Timing of genetic and cytological events. (**A**) Commitment to intragenic recombination. Wild-type MDY431×433 (*18*) (closed symbols) and an isogenic derivative homozygous for *ndj1*Δ::*TRP1*, MCY422×423 (open symbols), were shifted into sporulation medium at time zero. After the indicated intervals, samples were plated on selective media to assay gene conversion at heteroallelic loci, *HIS7* (circles) and *LEU1* (triangles), by production of prototrophs. Values are corrected for viability differences assayed by control platings on complete medium. Similar results were obtained with *LYS2* and *TYR1*. (**B** and **C**) Meiotic chromosome structures as analyzed by electron microscopy of silver-stained spread preparations (*11*). Fifty nuclei were examined per time point, and the percentage of those in or beyond the stage at which axial element formation (B) or synaptonemal complex formation (C) has initiated was determined. Values are slightly inflated by loss of more fragile vegetative cells during treatment with Zymolyase (ICN). (**D**) Cells that had completed the first meiotic division were identified by staining of whole cells with 4′,6′-diamidino-2-phenylindole. The percentage of cells in or beyond the stage at which chromosomes have divided into two distinct masses was determined. (B through D) Closed symbols, wild type; open symbols, *ndj1*Δ.

Table 1. Spore viability. The *ndj1*Δ::*TRP1* construct (*ndj1*Δ), which results in the removal of amino acids 14 to 252 from Ndj1p, was introduced into haploid strains MDY431 and MDY433 (*18*), which were then allowed to mate and sporulate. Spore viability was determined from dissected tetrads. Vb, viability.

Genotype	No. of tetrads	Tetrads with 0 to 4 viable spores (%)					Vb (%)
		0	1	2	3	4	
NDJ1	215	1	1	3	18	77	92
*ndj1*Δ	317	14	12	21	19	33	62

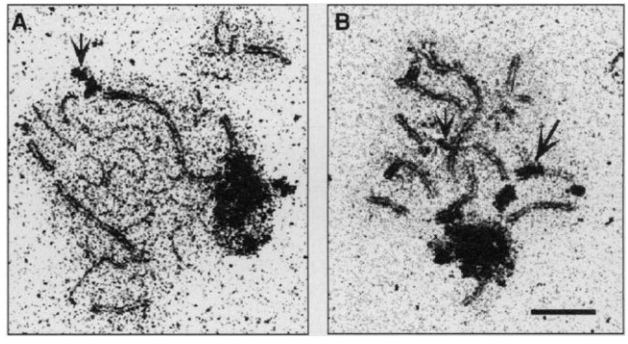

Fig. 2. Electron micrographs of silver-stained spread preparations (*11*). (**A**) Accumulation of unsynapsed axial elements (single lines) in an *ndj1*Δ cell, even though synapsis (paired lines separated by a uniform spacing) is complete for some chromosome pairs in this zygotene nucleus (6 hours after shift to sporulation medium). Complete duplication of the SPB, as shown here (small arrowhead), is normally accompanied by complete synapsis in wild-type cells. (**B**) Nuclei in *ndj1*Δ cells with apparently complete synapsis (pachytene) have polycomplexes (one of six indicated by larger arrowhead) (10 hours after shift to sporulation medium). Small arrowhead as in (A). Bar, 2 μm.

Table 2. Crossing-over and interference. *ndj1*Δ was introduced into haploid strains MDY431 and MDY433, which were then allowed to mate and sporulate as described in Table 1. Data from tetrads with four viable spores were used to calculate map distances [in centimorgans (cM)] on chromosomes VII, II, and V as indicated (*19*). Coefficients of crossover coincidence, a measure of interference, are presented between the map distances.

Genotype	No. of tetrads	VII					II			V
		LEU1-CYH2 (cM)	Interference	*CYH2-MET13* (cM)	Interference	*MET13-ADE5* (cM)	*LYS2-TYR1* (cM)	Interference	*TYR1-HIS7* (cM)	*CAN1-URA3* (cM)
NDJ1	165	56	0.17	15	0.08	62	48	0.19	60	38
*ndj1*Δ	106	49	0.31	14	0.15	83	43	0.10	60	53

II errors of homologous YACs were also observed in *ndj1Δ* cells (Table 3). In contrast to the behavior of linear YACs, distributive segregation of a pair of circular chromosomes was not impaired in *ndj1Δ* cells (Table 3).

Multiple cytological abnormalities were apparent in *ndj1Δ* cells. Initiation of the formation of axial elements (Fig. 1B) and of synapsis (synaptonemal complex formation) (Fig. 1C) was delayed, even though the kinetics of spindle pole body (SPB) duplication were similar to those of wild-type cells (*4*). Completion of synapsis was further delayed, resulting in abnormal nuclei with fully duplicated SPBs but incomplete synapsis (Fig. 2A) and, finally, in nuclei with apparently complete synapsis and polycomplexes (accumulations of synaptonemal complex material common in nuclei delayed in meiotic prophase) (Fig. 2B). Cells completing the first meiotic division appeared after a total delay of 4 to 6 hours (Fig. 1D). There was no abnormal accumulation of cells in the first division, and the second division was completed after the normal delay between the first and second divisions (*4*).

To determine the location of Ndj1p, we replaced the wild-type *NDJ1* gene with a version that encoded the hemagglutinin (HA) epitope at the 3′ end of the coding sequence. The extents of sporulation and spore viability for an *NDJ1-HA* homozygote were similar to those of the wild type. Spread preparations of meiotic nuclei (*11*) from an *NDJ1-HA* homozygote were stained with a monoclonal antibody to the HA epitope and visualized by indirect immunofluorescence. Ndj1p is abundant at telomeres (Fig. 3A), suggesting a telomere function even though it may function at other chromosomal locations at concentrations not readily detected by immunocytology, as do telomere proteins Rap1p, Sir3p, and Sir4p (*12*, *13*).

We examined telomeres directly with antibodies to Rap1p, which binds to sites in the telomeric DNA repeat sequence (*12*, *13*). Bivalent ends in wild-type pachytene nuclei (*11*) normally show a single spot of Rap1p (Fig. 3B). We detected 35.4 ± 4.0 spots per nucleus (n = 11), close to the expected value of 32 if the four telomeres at each bivalent end lie in close proximity. However, in *ndj1Δ* pachytene nuclei, ends were often marked by fainter or multiple smaller spots (Fig. 3, C and D) and nuclei averaged 68.9 ± 16.6 spots (n = 21), which was significantly different from the wild-type value (t test, $P < 0.001$) and indicates abnormal telomere organization. This observation is consistent with a failure in the formation or the persistence of stable associations between homologous or sister telomeres in *ndj1Δ* cells.

The *ndj1Δ* phenotype thus includes (i) delayed formation of axial elements, (ii) longer delays in the initiation of synapsis and in completion of the first meiotic division, (iii) a defect in telomere localization of Rap1p, (iv) an increased frequency of nondisjunction and PSS of homologs, (v) reduced levels of sporulation and spore viability, and (vi) defective distributive segregation of linear, but not circular, heterologs. Induction of meiotic recombination is similar to that in the wild type and the extent of crossing-over is largely normal. Localization of Ndj1p to the telomeres and the immunocytological and distributive segregation defects in *ndj1Δ* cells suggest that Ndj1p functions at telomeres, but it is possible that it also acts interstitially. Some or all of the defects in *ndj1Δ* cells may result

Table 3. Segregation of tester chromosomes. Data are the percentage of tetrads per class and, in parentheses, the actual number scored. The assays for segregation errors were performed as described with yeast strains containing homologous and heterologous linear human YACs (pairs A and F) (*8*). Each YAC contains a specific centromere marker whose segregation can be monitored relative to the segregation of a centromere marker on chromosome VI. Circular centromere plasmids were pMCB248, a randomly cloned 8.1-kb Bam HI fragment from chromosome XIII in pRS314, and pMCB249, a random 6.8-kb Bam HI fragment from chromosome XIV in pRS316. Their segregation was assayed in the same strain background as for the linear YACs. The classes of segregation errors are (i) nondisjunction, both YACs or plasmids go to the same pole at the first division; (ii) PSS (precocious sister separation), sister chromatids of one YAC or plasmid segregate to opposite poles at meiosis I; (iii) PSS×2 (double precocious sister separation), sister chromatids from both YACs or plasmids separate prematurely; and (iv) abnormal meiosis II, chromosome loss or nondisjunction at the second division results in one pair of sister spores with normal segregation and the other pair with one member missing a YAC or plasmid. Significant effects (G test) of *ndj1Δ* were detected for the disjunction of heterologs [G = 9.97; 1 degree of freedom (df); $P < 0.005$], combined PSS and PSS×2 of homologs (G = 4.53; 1 df; $P < 0.05$), and meiosis II errors of homologs (G = 3.75; 1 df; P = 0.05).

Type of division		Linear				Circular	
		Homologous		Heterologous			
		NDJ1	*ndj1Δ*	*NDJ1*	*ndj1Δ*	*NDJ1*	*ndj1Δ*
Meiosis I*	Disjunction	100 (45)	99 (106)	89 (34)	62 (43)	80 (28)	86 (55)
	Nondisjunction	0 (0)	1 (1)	11 (4)	38 (26)	20 (7)	14 (9)
Meiosis I	Non-PSS	100 (45)	95 (107)	83 (38)	73 (69)	75 (35)	80 (64)
	PSS	0 (0)	4 (5)	15 (7)	17 (16)	23 (11)	19 (15)
	PSS×2	0 (0)	1 (1)	2 (1)	10 (9)	2 (1)	1 (1)
Meiosis II	Normal	98 (44)	89 (101)	91 (42)	89 (84)	89 (42)	85 (68)
	Abnormal	2 (1)	11 (12)	9 (4)	11 (10)	11 (5)	15 (12)
Total tetrads		45	113	46	94	47	80

*Excludes tetrads with PSS or PSS×2.

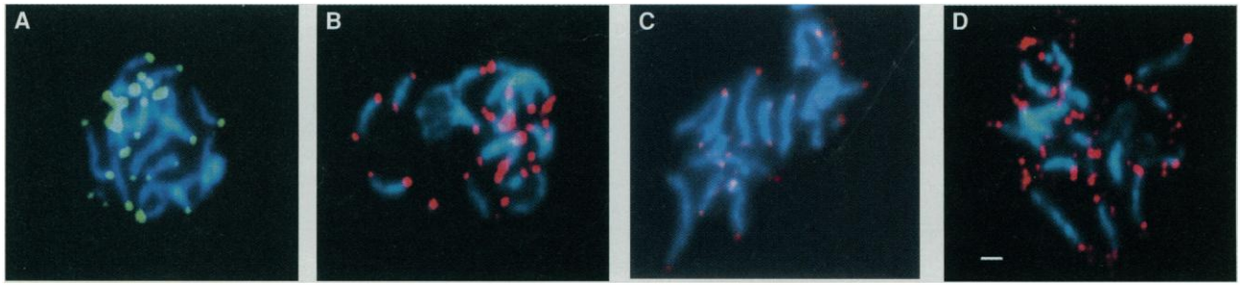

Fig. 3. Immunocytological localization of Ndj1p and analysis of telomere behavior. (**A**) Pachytene nucleus from an *NDJ1-HA* homozygote labeled with monoclonal antibody 12CA5 (Babco) to the HA epitope (yellow-green staining). No fluorescence signal is apparent in nuclei not containing the HA epitope under these conditions. (**B** through **D**) Pachytene nuclei from wild-type (B) and *ndj1Δ* (C and D) cells labeled with antibodies to Rap1p (red staining). Blue staining in (A) through (D) represents 4′,6′-diamidino-2-phenylindole–stained DNA. Bar in (D), 1 μm.

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.

REFERENCES AND NOTES

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).
2. M. Vandenbol *et al.*, *Yeast* **11**, 1069 (1995).
3. N. Burns *et al.*, *Genes Dev.* **8**, 1087 (1994).
4. M. N. Conrad, A. M. Dominguez, M. E. Dresser, unpublished results.
5. M. Goldway, T. Arbel, G. Simchen, *Genetics* **133**, 149 (1993); M. Flatters and D. Dawson, *ibid.* **134**, 423 (1993).
6. R. S. Hawley, in *Genetic Recombination*, R. Kucherlapati and G. R. Smith, Eds. (American Society for Microbiology, Washington, DC, 1988), pp. 497–528.
7. T. D. Petes, R. E. Malone, L. S. Symington, in *The Molecular and Cellular Biology of the Yeast* Saccharomyces: *Genome Dynamics, Protein Synthesis, and Energetics*, J. R. Broach, J. R. Pringle, E. W. Jones, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, ed. 2, 1991), vol. 1, pp. 407–521.
8. D. D. Sears, J. H. Hegemann, P. Hieter, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5296 (1992).
9. D. D. Sears, P. Hieter, G. Simchen, *Genetics* **138**, 1055 (1994).
10. L. Clarke and J. Carbon, *Nature* **287**, 504 (1980); C. Mann and R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6017 (1986); D. S. Dawson, A. W. Murray, J. W. Szostak, *Science* **234**, 713 (1986); T. Nilsson-Tillgren *et al.*, *Carlsberg Res. Commun.* **51**, 309 (1986); D. B. Kaback, *Curr. Genet.* **15**, 385 (1989); J. Loidl, H. Scherthan, D. B. Kaback, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 331 (1994).
11. M. E. Dresser and C. N. Giroux, *J. Cell Biol.* **106**, 567 (1988).
12. F. Klein *et al.*, *ibid.* **117**, 935 (1992).
13. M. Gotta *et al.*, *ibid.* **134**, 1349 (1996).
14. B. M. Weiner and N. Kleckner, *Cell* **77**, 977 (1994).
15. M. P. Maguire, *Exp. Cell Res.* **112**, 297 (1978).
16. K. E. Kirk, B. P. Harmon, I. K. Reichardt, J. W. Sedat, E. H. Blackburn, *Science* **275**, 1478 (1997).
17. M. J. Moses, *Annu. Rev. Genet.* **2**, 363 (1968).
18. M. E. Dresser *et al.*, *Genetics* **138**, 633 (1994).
19. D. D. Perkins, *ibid.* **34**, 607 (1949).

20. We thank C. Barton, D. Coody, D. Ewing, and J. Hulen for technical assistance; P. Hieter, H. Ronne, and K. Runge for strains and plasmids; J. Berman for antibodies to Rap1p; P. Silverman for reading the manuscript; and the core facility at the University of Oklahoma Health Sciences Center for oligonucleotides. Supported by grants GM45250 from NIH to M.E.D., HN2-034 from the Oklahoma Center for the Advancement of Science and Technology to M.N.C., and MCB-9507089 from NSF to M.E.D. and M.N.C.

10 November 1996; accepted 25 March 1997

t-SNARE Activation Through Transient Interaction with a Rab-Like Guanosine Triphosphatase

Vladimir V. Lupashin and M. Gerard Waters*

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

Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA.

*To whom correspondence should be addressed. E-mail: gwaters@molbiol.princeton.edu