JOURNAL OF BACTERIOLOGY, Jan. 1984, p. 334–336
0021-9193/84/010334-03$02.00/0

Vol. 157, No. 1

A Meiotic Mutant of the Fission Yeast *Schizosaccharomyces pombe* That Produces Mature Asci Containing Two Diploid Spores

YUKINOBU NAKASEKO, OSAMI NIWA, AND MITSUHIRO YANAGIDA*

Department of Biophysics, Faculty of Science, Kyoto University, Sakyo-ku, Kyoto 606, Japan

Received 17 May 1983/Accepted 4 October 1983

A mutant of the fission yeast *Schizosaccharomyces pombe* grew normally in the mitotic cycle but produced two-spored asci in the meiosis cycle. These spores were diploid, and the segregation of centromere-linked markers in the dyads was mostly reductional. Only the first meiotic division appears to occur in this *tws1* mutant, resulting in enclosure of diploid nuclei into spores.

Meiosis in general includes meiotic recombination and two successive nuclear divisions to yield four haploid cells. In the budding yeast *Saccharomyces cerevisiae*, mutants exist which produce asci containing two diploid ascospores (4, 7). Recombination and chromosome segregation during the single-division meiosis in two such mutants, *spo12-1* and *spo13-1*, were studied, and the segregation of centromere-linked markers in the two-spored (dyad) products indicated that the division is generally equational, that is, a type of the second meiotic division (8). In the fission yeast *Schizosaccharomyces pombe*, several meiosis-defective mutants (*mei* and *mes*) have been isolated (2; C. Shimoda, personal communication), but no mutant producing two spores has been reported. In this paper isolation of such a mutant and its properties are described.

Genetical procedures described by Gutz et al. (5) were followed. A wild-type homothallic strain of *S. pombe* (h^{90}) was treated with *N*-methyl-*N'*-nitro-nitrosoguanidine. During the course of experiments for isolation of meiosis-negative mutants, a strain (N22) that forms asci containing only two spores was found. The mutant cells grew normally (generation time, 120 to 130 min at 30°C) in a rich YPD medium (per liter: 20 g of polypeptone, 10 g of yeast extract, 20 g of glucose). On transfer to a sporulation medium limited in nitrogen sources, zygotes and asci were formed as efficiently as wild type. However, ca. 80 to 90% of the asci contained only two spores (Fig. 1). The rest had four spores, each of which retained the two-spored phenotype. Zygotes produced by a cross between wild-type strain h^{90} and the mutant produced 100% four-spored asci, indicating that the mutation is recessive. A total of 17 tetrads were analyzed, and all of them showed 2:2 segregation for the two-spored phenotype. A single gene designated as *tws1* (*two-s*pored) seems to be responsible for production of the dyads. Haploid *tws1*-N22 maintained on MR plates (5) was used in the following experiments.

The *tws1* mutation was introduced into heterothallic strains (h^- and h^+), and to determine the segregation pattern, three crosses were carried out as follows: cross I, between h^+ *his2 cyh1 tws1* and h^- *ade6*-M210 *tws1*; cross II, between h^+ *his2 cyh1 tps13*-24 *tws1* and h^- *ade6*-M210 *arg5 tws1*; and cross III, between h^+ *his2 cyh1 tws1* and h^- *lys1 tws1*. Among the genetic markers used (all recessive), those that were centromere-linked were *cyh1*, *lys1* (chromosome I), *tps13*-24 (chromosome II), and *ade6*-M210 (chromosome III). The markers, *his2* (chromosome II) and *arg5* (chromosome II), were unlinked to centromeres (9) (Fig. 1).

The results of the dyad analyses are shown in Table 1. For crosses I, II, and III, a total of 35, 80, and 81 dyads, respectively, were analyzed. Two classes of the segregation patterns were observed; the marker phenotype of a dyad was either $1^+:1^-$ (presumed genotype, +/+ and −/−) or $2^+:0^-$ (presumed genotype, +/− and +/−). The former was defined as reductional, and the latter was defined as equational. The segregation was mostly reductional for the markers tightly linked to centromeres. Only 7.1% (14 of 196), 7.4% (6 of 81) and 0% (0 of 80) of the dyads were equational for *cyh1*, *lys1*, and *tps13*, respectively. For weakly centromere-linked *ade6*, 28% (32 of 115) of the total dyads were equational. On the other hand, for the markers not linked to centromeres (*his2* and *arg5*), roughly half of the dyads were equational. The increase of the dyads with the equational segregation pattern was found to approximately correspond to the distances of the markers from the centromeres (9).

Furthermore, in cross III all of the dyads with equational segregation for *lys1* were also equational for *cyh1*. One dyad was found to be equational for *cyh1* but reductional for *lys1*. These results may suggest an order of centromere-*lys1*-*cyh1* in chromosome I, although the direction of these genes in one of the two arms remains to be determined. Thus the mutant may be useful for rapid and detailed mapping near centromeres.

Spores in the dyads appear to be diploid based on the following evidence. Spores (4.0 by 4.9 μm) in the dyads were much larger than the haploid spores (2.7 by 2.7 μm). Vegetative cells (4.7 by 24.5 μm) germinated from the dyads were also larger than haploid cells (3.5 by 10 μm). In all of the dyads showing the $2^+:0^-$ segregation pattern for *his2* in the crosses shown in Table 1, both of the spores could produce asci without conjugation, indicating that their mating type locus (closely linked within 1 cM to *his2*) was heterozygous. Occasionally, *ade6*$^-$ colonies (red) were segregated out of Ade$^+$ colonies (white) derived from the dyads with the $2^+:0^-$ segregation about the locus. Germination frequency of the dyads was high (more than 90%). The germinated cells were stable in their genetic markers. Since aneuploid spores of *S. pombe* were unstable and poor in germination (9; O. Niwa, unpublished results), it is unlikely that the dyads contained aneuploid spores.

Spores of *tws1* were stained with a DNA-specific fluores-

* Corresponding author.

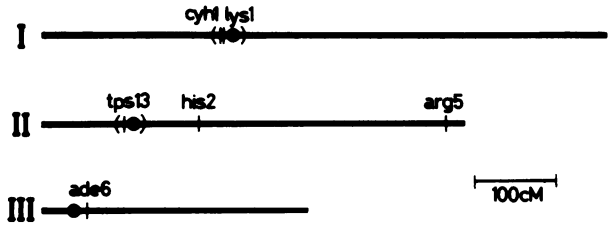

FIG. 1. Map positions of the markers used on three chromosomes of *S. pombe*. ●, Centromeres. Actual map positions of *cyh1*, *lys1*, and *tps13* relative to centromeres are much closer than those indicated in the figure (see text).

cent dye, 4',6-diamidino-2-phenylindole (13). Only a single domain in the spores was found to be stained, indicating that the spores contain a single nucleus. Sporulating cells of the mutant in the sporulation medium were also stained with 4',6-diamidino-2-phenylindole. Most of the zygotes were found to contain only two 4',6-diamidino-2-phenylindole-stainable bodies, whereas wild-type sporulating cells showed four 4',6-diamidino-2-phenylindole-staining bodies.

The above results suggest that, in the presence of the *tws1* mutation, only the first reductional division takes place, producing a pair of diploid nuclei that are eventually enclosed into viable spores. The second meiotic division seems to be nonessential for spore formation. It should be pointed out that in *S. pombe* the first division is accompanied with separation of the nuclear membranes (6, 14); the formation of the forespore membranes has already started at the two-nucleated stage. This is strikingly different from *S. cerevisiae*, in which the nuclear membranes separate only after the second division (10).

The gene function of *tws1* may be involved specifically at a stage in the second meiotic division, and its defect causes the absence of the equational division but allows spore formation. Two previously isolated mutants in *mes1* and *mes2* accumulate the two-nucleated zygotes that are incapable of sporulation (2; C. Shimoda, personal communication). Hence, these genes should differ from *tws1*. In fact, double mutants (*mes1 tws1* and *mes2 tws1*) were constructed and were found to produce only the two-nucleated zygotes but no spores, indicating that *mes1* and *mes2* are epistatic to

TABLE 1. Segregation patterns in dyads formed by *tws1*-N22 mutation

Segregation pattern	Markers					
	cyh1	*lys1*	*tps13*	*ade6*	*his2*	*arg5*
Cross I[a]						
$1^+:1^-$	32			30	13	
$2^+:0^-$	3			5	22	
Cross II[b]						
$1^+:1^-$	76		80	53	34	33
$2^+:0^-$	4		0	27	46	47
Cross III[c]						
$1^+:1^-$	74	75				
$2^+:0^-$	7	6				

[a] h^+ *his2 cyh1 tws1* × h^- *ade6 tws1*.
[b] h^+ *his2 cyh1 tps13 tws1* × h^- *ade6 arg5 tws1*.
[c] h^+ *his2 cyh1 tws1* × h^- *lys1 tws1*. *cyh1*, *lys1*, and *tps13* are tightly linked to centromeres, whereas *ade6* is weakly linked. *his2* and *arg5* are not linked to centromeres.

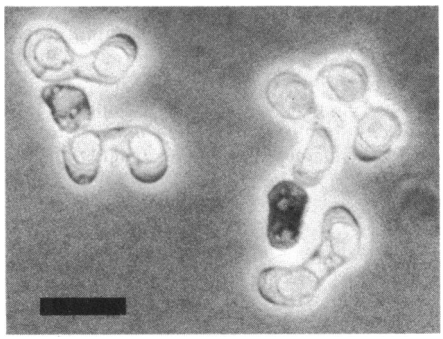

FIG. 2. Phase-contrast micrograph of two-spored asci produced by mutant *tws1*-N22. Bar, 10 μm.

tws1. Some of nuclear division arrest *cdc* mutants of *S. pombe* such as *cdc2* and *nda3* have been observed to produce two-spored asci at or near the restrictive temperature (R. Egel, personal communication; Y. Nakaseko, unpublished results). However, viability of the spores and the segregation pattern have not been examined. In *S. cerevisiae*, two mutants (*cdc5* and *cdc14*) with a temperature-sensitive defect during mitosis were shown to produce asci containing two diploid spores during meiosis at a semirestrictive temperature, in which centromere-linked markers segregated in the reductional manner (12). Meiotic mutants producing dyads with the reductional segregation were found in plants such as *Citrus* and *Datura* (1, 3, 11).

We thank C. Shimoda and R. Egel for strains and helpful comments.

This work was supported by grants from the Ministry of Education, Science and Culture of Japan.

LITERATURE CITED

1. **Baker, B. S., A. T. C. Carpenter, M. S. Esposito, R. E. Esposito, and L. Sandler.** 1976. The genetic control of meiosis. Annu. Rev. Genet. **10**:53–134.
2. **Bresch, C., G. Muller, and R. Egel.** 1968. Genes involved in meiosis and sporulation of a yeast. Mol. Gen. Genet. **102**:301–306.
3. **Esen, A., R. K. Soost, and G. Geraci.** 1979. Genetic evidence for the origin of diploid megagametophytes in *Citrus*. J. Hered. **70**:5–8.
4. **Grewal, N. S., and J. J. Miller.** 1972. Formation of asci with two diploid spores by diploid cells of *Saccharomyces*. Can. J. Microbiol. **18**:1897–1905.
5. **Gutz, H., H. Heslot, U. Leupold, and N. Loprieno.** 1974. *Schizosaccharomyces pombe*. p. 395–446. *In* R. C. King (ed.), Handbook of genetics, vol. 1. Plenum Publishing Corp., New York.
6. **Hirata, A., and K. Tanaka.** 1982. Nuclear behavior during conjugation and meiosis in the fission yeast *Schizosaccharomyces pombe*. J. Gen. Appl. Microbiol. **28**:263–274.
7. **Klapholz, S., and R. E. Esposito.** 1980. Isolation of *spo12-1* and *spo13-1* from a natural variant of yeast that undergoes a single meiotic division. Genetics **96**:567–588.
8. **Klapholz, S., and R. E. Esposito.** 1980. Recombination and chromosome segregation during the single division meiosis in *spo12-1* and *spo13-1* diploids. Genetics **96**:589–611.
9. **Kohli, J., H. Hotting, P. Munz, A. Strauss, and P. Thuriaux.** 1977. Genetic mapping in *Schizosaccharomyces pombe* by mitotic and meiotic analysis and induced haploidization. Genetics **87**:471–489.
10. **Moens, P. B., and E. Rapport.** 1971. Spindles, spindle plaques and intranuclear meiosis in the *Saccharomyces cerevisiae*. J. Cell Biol. **50**:344–361.

11. **Satina, S., and A. F. Blakeslee.** 1935. Cytological effects of a gene in *Datura* which causes dyad formation in sporogenesis. Bot. Gaz. (Chicago) **96**:521–532.
12. **Schild, D., and B. Byers.** 1980. Diploid spore formation and other meiotic effects of two cell-division-cycle mutants of *Saccharomyces cerevisiae*. Genetics **96**:859–876.
13. **Toda, T., M. Yamamoto, and M. Yanagida.** 1981. Sequential alterations during mitosis in the fission yeast *S. pombe*: video fluorecent microscopy of synchronously growing wild type and cold sensitive *cdc* mutants by using a DNA-binding fluorescent probe. J. Cell Sci. **52**:271–287.
14. **Yoo, B. Y., G. B. Calleja, and B. F. Johnson.** 1973. Ultrastructural changes of the fission yeast (*Schizosaccharomyces pombe*) during ascospore formation. Arch. Mikrobiol. **91**:1–10.