Identification of seven new cut genes involved in *Schizosaccharomyces pombe* mitosis

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SUMMARY

Fission yeast cut mutants cause cytokinesis in the absence of normal nuclear division. These mutants show abnormal uncoupled mitosis and are known to be the result of mutations in the genes encoding DNA topoisomerase II, proteins related to spindle pole duplication, and a kinesin-related mitotic motor. We have screened 717 temperature-sensitive (ts) mutants by individually observing their cytological phenotypes at the restrictive temperature, and have newly isolated 25 cut mutants. Genetic analyses indicate that 14 of them fall into five previously identified loci, namely, *top2*, *cut1*, *cut5*, *cut7* and *cut9*, whereas nine have been mapped onto seven new loci, designated *cut13* to *cut19*. The cytological phenotypes of the newly identified cut mutants can be classified into three groups. One group consists of mutants in which a portion of the nuclear chromatin is stretched by the elongated spindle but the entire nucleus is not separated, reminiscent of, but not identical to, the phenotypes of *top2* and *cut1*; mutants *cut14-208*, *cut15-85*, *cut16-267* and *cut17-275* display such a phenotype. Another group exhibits non-disjunctioned and condensed chromosomes in the presence of the spindle; *cut13-131* belongs to this group. The *cut19-708* mutant has also been found to have condensed chromosomes. The remaining group has a mixed phenotype of the above two groups; namely, stretched chromatin and condensed chromosomes; *cut18-447* exhibits such a phenotype. The isolation and characterization of the mutated genes will be the subjects of future investigations.

Key words: mitosis, cut mutant, fission yeast, DNA topoisomerase II, motor protein, spindle movement

INTRODUCTION

A set of duplicated chromosomes is separated and distributed to each of two daughter cells during mitosis and subsequent cytokinesis. Nuclear division consists of a number of processes that include chromosome condensation, spindle formation, kinetochore attachment to the spindle, sister chromatid separation and chromosome movement to the poles, followed by spindle elongation and chromosome decondensation. To ensure high-fidelity transmission of genetic information to daughter cells, these processes must be executed in a precise and coordinated fashion. Failure of any of these processes would lead to a disorganized mitosis and daughter cells with altered genetic information. Such a situation is generally a lethal event.

One approach to the investigation of the mitotic process is to isolate and characterize mutants. Many mitotic mutants of the fission yeast *Schizosaccharomyces pombe* have been isolated and have proved to be useful for understanding the control mechanism of mitosis (Nurse, 1990; Yanagida, 1989). A group of temperature-sensitive (ts) mutants called cut (cell untimely rom; Hirano et al., 1986) are of particular interest with regard to coordination of different mitotic events; cut mutants do not arrest at a specific stage at the restrictive temperature (36°C), but undergo uncoordinated mitosis, i.e. cytokinesis takes place without prior completion of nuclear division, resulting in uncoordinated cleavage of an undivided nucleus by the septum (Fig. 1). In some cut mutants, cells containing the septum and the displaced nucleus also appear, in addition to the cells with the cut phenotype.

Mutations in a variety of genes are known to produce the cut phenotype (Fig. 1). *top2* mutants, defective in DNA topoisomerase II (Uemura and Yanagida, 1984), show a typical uncoordination between nuclear division and cytokinesis, so causing this phenotype (Fig. 1; Uemura and Yanagida, 1986). A small portion of the nuclear chromatin is pulled by the transient mitotic spindle in *top2* mutant cells at 36°C. In *cut1* and *cut2* mutants, a large portion of the nuclear chromatin is extended by apparently normal spindle dynamics, but the nuclear chromatin moves back to the middle of the cell after the spindle has broken down (Uzawa et al., 1990); cytokinesis follows the cut across the undivided nucleus. Polyploid chromosomes are formed in *cut1* and *cut2* if septation is blocked (Uzawa et al., 1990; Creanor and Mitchison, 1990). The product of the *cut1*
gene partly resembles that of the budding yeast ESP1 gene (Baum et al., 1988). The cut7+ gene was isolated by transformation and its predicted product has an amino acid sequence similar to that of the microtubule-dependent motor, kinesin (Hagan and Yanagida, 1990). cut7 mutants are defective in spindle formation, probably caused during the process of interdigitation of two half-spindles (Hagan and Yanagida, 1992). cut9 mutant cells show a temporal arrest in metaphase at 36°C (Fig. 1; I. Samejima and M. Yanagida, unpublished result). The cut9+ gene was cloned by transformation and the gene product has been characterized (I. Samejima and M. Yanagida, unpublished).

Nine cut genes (cut1-cut9+) were originally identified (Hirano et al., 1986) and two other genes (cut11+ and cut12+) were recently found (R. Bartlett and P. Nurse, personal communication) so that 11 genes showing the cut phenotype have so far been identified (the cut10 locus was found to be identical to cut1; Uzawa et al., 1990, and S. Uzawa, unpublished result). With regard to the phenotypes that cause a fatal event in uncoupled mitosis, potentially these genes have the ability to monitor a checkpoint event in cell cycle control (Weinert and Hartwell, 1988; Hartwell and Weinert, 1989). None of the cut+ genes, including top2+ has, however, been shown to have such a function. Interestingly, fission yeast checkpoint-deficient mutants, isolated because of their inability to grow in the presence of hydroxyurea, a DNA synthesis inhibitor, display the cut phenotype (Enoch et al., 1991); these mutants enter mitosis without completing DNA synthesis, in the presence of hydroxyurea. Four checkpoint mutants (rad1, rad3, rad9 and rad17) have been identified; these mutants also enter abnormal mitoses when cells are either irradiated with gamma-rays or blocked in DNA synthesis (Rowley et al., 1992; Al-khodairy and Carr, 1992).

In the present study, a new search for cut mutants has been made using a ts mutant collection, which was used for the isolation of mutants that produce condensed chromosomes at the restrictive temperature (Matsumoto and Beach, 1991). Genetic analyses of the isolated mutants have identified seven new cut loci that produce distinct phenotypes.

MATERIALS AND METHODS

Schizosaccharomyces pombe strains used were 975h+, JY6 (h+ leu1 his2), SP6/HM123 (h- leu1), cut1-cut9 (Hirano et al., 1986), top2 (Uemura and Yanagida, 1984), sad1-549 (Hagan and Yanagida, 1990), dip7, cut11, and cut12 (gifts from R. Bartlett and P. Nurse). Standard genetic procedures for tetrad dissection, random spore and heterozygous diploid analyses, as described by Gutz et al. (1974), were followed.

Media used were YEA (0.5% yeast extract, 3% glucose), YPD (1% yeast extract, 2% polypeptone, 2% glucose), SD (minimal medium; 0.67% yeast nitrogen base without amino acids, 2% glucose; 1.7% agar was added for plates), and EMM2 (minimal medium; Mitchison, 1970). Media containing 1.6% agar were used for plating, unless indicated otherwise. SPA medium contained (per l): 10 g dextrose, 1 g KH2PO4, 10 mg biotin, 1 mg calcium pantothenate, 10 mg nicotinic acid, 10 mg meso-inositol and 30 g agarose and was used for sporulation. Escherichia coli was grown in LB (0.5% yeast extract, 1% polypeptone, 1% NaCl, (pH7.5); 1.5% agar was added for plates).

The procedure for mutagenesis was described in detail by Matsumoto and Beach (1991). Mutant screening for the cut phenotype was carried out by growing individual ts strains in YEA or YPD at the permissive temperature, and then incubating at the restrictive temperature. All the ts strains isolated and used for backcrossing showed the ts phenotype in both YPD and YEA media, except for strains 599 and 708 where the phenotype was not as evident in YPD.

Fluorescence microscopy

The procedure for DAPI staining (4',6-diamidino-2-phenylindole) staining of S. pombe cells, described by Adachi and Yanagida (1989) was followed. Immunofluorescence microscopy using anti-tubulin antibody (TAT1, a gift from Dr. K. Gull; Woods et al., 1989) and...
aldehyde fixation was described by Hagan and Hyams (1988). The secondary antibody used was FITC-conjugated goat anti-mouse antibody (E.Y. Lab. Inc.). Each ts mutant cell was first grown in YPD medium at the permissive temperature (26°C), and then transferred to the restrictive temperature. Cells were taken at one-hour intervals and observed under an epifluorescence microscope after DAPI staining.

RESULTS

Isolation of cut mutants

A haploid S. pombe strain, SP6 (h− leu1), was mutagenized by nitrosoguanidine, and ts mutants were selected by replica-plating at the permissive (26°C) and restrictive (36°C) temperatures. One thousand ts mutants were thus isolated, and 717 were subjected to cytological screening (Matsumoto and Beach, 1991). Each strain grown at 26°C was transferred to 36°C for 4 h prior to DAPI staining. Cells were observed by fluorescence microscopy, which revealed the nuclear chromatin structure within mutant cells (Toda et al., 1981; Uemura and Yanagida, 1984; Hirano et al., 1986; Ohkura et al., 1988). Twenty-five mutant strains, which showed the cut phenotype (Hirano et al., 1986) in more than 5% of their cell population at 36°C, were chosen for further study.

Allelism test among cut mutants

Twenty-five newly identified cut mutants were first backcrossed with the wild-type h+ strain. Four (523, 666, 668, 676) were found to be sterile and failed to conjugate with the wild type. In the remaining twenty-one strains, the ts phenotype co-segregated 2+;2−, shown by tetrad analysis; hence single mutations are responsible for the ts lethality in these strains. One exception was the ts phenotype of cut19-708, which was not segregated 2+;2−, so that it might not be due to a single mutation.

Thirteen mutants that produced the cut phenotype were previously identified, namely, top2 (Uemura and Yanagida, 1984), cut1-cut9 (Hirano et al., 1986), cut11 and cut12 (R. Bartlett and P. Nurse, personal communication) and sad1 (I. Hagan and M. Yanagida, unpublished). These were crossed with the 21 new cut mutant strains, and a random spore analysis for the ts phenotype was carried out. Unless two ts− mutations in the crossed strains are tightly linked, about a quarter of the progeny spores will be the wild-type recombinants (ts+). The recombination frequency between mutations in an identical gene will be less than 0.05%. As shown in Table 1, twelve of the twenty-one mutants examined fell into the previously identified five loci: cut1 was allelic to five strains (380, 474, 514, 686, and 693); top2 to three (4, 137, and 640); cut7 to two (138 and 611); and two strains to cut5 (401) and cut9 (98), respectively. The other nine strains (85, 122, 131, 208, 267, 275, 447, 599, and 708) were linked to none of them.

To confirm the above assignments, plasmids carrying the corresponding wild-type genes were introduced into mutant cells and examined to determine whether the mutant phenotypes could be suppressed by a plasmid carrying the gene tightly linked to the mutation site. The five genes used have previously been cloned and characterized (top2+, Uemura et al., 1986; cut1+, Uzawa et al., 1990; cut7+, Hagan and Yanagida, 1990; cut5+, Y. Saka and M. Yanagida, unpublished result; cut9+, I. Samejima and M. Yanagida, unpublished result). These plasmids were introduced into five representative mutant strains by transformation. We found that they were able to suppress the ts phenotype of corresponding mutants, therefore supporting the above genetic identifications.

The four sterile strains were also transformed by the five plasmids. Temperature sensitivity, but not sterility, of strains 668 and 676 was rescued by plasmids carrying the top2+ and cut1+ genes, respectively. This indicates that the cut phenotypes of 668 and 676 are due to mutations in the top2+ and cut1+ genes, respectively. None of the plasmids rescued the ts lethal phenotype of the other two sterile strains, 666 and 523. Therefore, we concluded that fourteen newly isolated ts cut mutants were derived from five known genetic loci.

Table 1. Random spore analyses of twenty-one newly isolated cut mutants

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Table 2. Pairwise crosses among nine new cut mutants

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Identification of seven new cut genes

The remaining nine strains were crossed with JY6 (h* leu1 his2), then subjected to complementation (criss-cross) tests using the 81 possible pairwise crosses. As shown in Table 2, strains 85 and 122, and 131 and 599, respectively, were found to belong to the same locus, but the other five showed no linkage to any strains. Thus these nine mutations constituted seven new complementation groups.

The new cut loci were designated cut13 (131, 599), cut14 (208), cut15 (85, 122), cut16 (267), cut17 (275), cut18 (447) and cut19 (708). Heterozygous diploids were made for each cut mutant by crossing it with a mei1 mutant, and their ts phenotype was examined. All of them were found to be ts+, so these new cut mutations were recessive.

The frequencies of the cut phenotype in strains 85 and 122, and 131 and 599, respectively, were found to belong to the same locus, but the other five showed no linkage to any strains. Thus these nine mutations constituted seven new complementation groups.

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None of the cut mutants so far isolated has shown this mitotic defect. The short spindle phenotype and the absence of sister chromatid separation somewhat resembled the phenotypes of cut4, cut8 and cut9 mutants (I. Samejima and M. Yanagida, unpublished result).

Characterization of the cut14-208 mutant

The frequency of interphase cells in cut14-208 (indicated by filled circles in Fig. 3A) decreased, while cells with the cut phenotype (open circles) or septate cells (filled squares) increased to 58% and 16%, respectively, when mutant cells were incubated at 36°C for 4 h. In addition, cells displaying the condensed chromosomes (open circles; Fig. 2D) peaked (maximal level, 12%). Cells with the cut phenotype (open circles; Fig. 2D) increased after 2 h and reached 38% after 4 h. In addition, septate cells containing an undivided nucleus (filled squares; Fig. 2E) were seen in abundance. The frequency of such cells began to increase after 3 h and attained 45% after 4 h. Hence, approximately 83% cells of cut13-131 were abnormal in septation and cytokinesis at 36°C after 4 h.

To determine whether the spindle was formed in cut13-131 cells at 36°C, cells were stained by the anti-tubulin antibody TAT1 (Woods et al., 1989) for immunofluorescence microscopy. As shown in Fig. 2F, cells with a short spindle (average length 2 µm) showed condensed chromosomes arranged in a metaphase plate-like structure (Hirano et al., 1988). Aster microtubules (Hagan and Hyams, 1988) were also seen. The frequency of such mid-mitotic cells peaked after 1 h at 36°C. Cells with a longer spindle were also seen, but infrequently. The length of the longer spindles was 4-6 mm, still much shorter than the wild-type elongated spindle (maximum 15 µm). In those cells containing the longer spindle, condensed chromosomes were dissociated and scattered along the spindle in the absence of sister chromatid separation (Fig. 2G). Thus, the spindle formed but failed to fully elongate. However, the spindle structures were not visible in cells after 3 h at 36°C; instead, the cytoplasmic microtubule arrays were abundant in septate cells.

The cellular and nuclear structures of cut13-131 at 36°C were observed by fluorescence microscopy. Mutant cells were first exponentially grown at 26°C, and then transferred to 36°C. Samples of the cultures were withdrawn at hourly intervals, and cells were fixed with glutaraldehyde, stained with DAPI and observed under a fluorescence microscope. The frequency of interphase cells in cut13-131, cut14-208, and cut15-85, 35-60% cells showed the cut phenotype at 36°C for 4 h. However, in cut16-267 and cut17-275, the frequencies were 10-20%.

Characterization of the cut13-131 mutant

The cellular and nuclear structures of cut13-131 at 36°C were observed by fluorescence microscopy. Mutant cells were first exponentially grown at 26°C, and then transferred to 36°C. Samples of the cultures were taken at one-hour intervals, fixed by glutaraldehyde, stained by DAPI and observed under an epi-fluorescence microscope. The wild-type interphase cells showed a single nucleus displaying the hemispherical chromatin region, while mitotic cells displayed condensed chromosomes or dividing nuclei (Toda et al., 1981). After nuclear division, cells underwent septation, followed by cell separation (Fig. 1).

As shown in Fig. 2A, a culture of cut13-131 initially contained a high proportion (80%) of normal interphase cells (indicated by filled circles; also see Fig. 2B). The frequency of the interphase cells decreased with the time of incubation at 36°C, down to a level of 10% after 4 h. One hour after transfer to 36°C, cells displaying the condensed chromosomes (open triangles; Fig. 2C) peaked (maximal level, 12%). Cells with the cut phenotype (open circles; Fig. 2D) increased after 2 h and reached 38% after 4 h. In addition, septate cells containing an undivided nucleus (filled squares; Fig. 2E) were seen in abundance. The frequency of such cells began to increase after 3 h and attained 45% after 4 h. Hence, approximately 83% cells of cut13-131 were abnormal in septation and cytokinesis at 36°C after 4 h.

To determine whether the spindle was formed in cut13-131 cells at 36°C, cells were stained by the anti-tubulin antibody TAT1 (Woods et al., 1989) for immunofluorescence microscopy. As shown in Fig. 2F, cells with a short spindle (average length 2 µm) showed condensed chromosomes arranged in a metaphase plate-like structure (Hirano et al., 1988). Aster microtubules (Hagan and Hyams, 1988) were also seen. The frequency of such mid-mitotic cells peaked after 1 h at 36°C. Cells with a longer spindle were also seen, but infrequently. The length of the longer spindles was 4-6 mm, still much shorter than the wild-type elongated spindle (maximum 15 µm). In those cells containing the longer spindle, condensed chromosomes were dissociated and scattered along the spindle in the absence of sister chromatid separation (Fig. 2G). Thus, the spindle formed but failed to fully elongate. However, the spindle structures were not visible in cells after 3 h at 36°C; instead, the cytoplasmic microtubule arrays were abundant in septate cells.

None of the cut mutants so far isolated has shown this mitotic defect. The short spindle phenotype and the absence of sister chromatid separation somewhat resembled the phenotypes of cut4, cut8 and cut9 mutants (I. Samejima and M. Yanagida, unpublished result).

Characterization of the cut14-208 mutant

The frequency of interphase cells in cut14-208 (indicated by filled circles in Fig. 3A) decreased, while cells with the cut phenotype (open circles) or septate cells (filled squares) increased to 58% and 16%, respectively, when mutant cells were incubated at 36°C for 4 h. In addition, cells displaying a portion of nuclear chromatin apparently pulled by the mitotic spindle (open squares) gradually increased and peaked at a level of 25% after 3 h (DAPI stained cells; Fig. 3B, top). The frequency of cells containing such an abnormal mitotic nuclear structure decreased to 4% after 4 h. Cytokinesis was often seen to cut across the undivided nucleus.

Anti-tubulin antibody TAT1 staining showed that the mitotic spindle fully elongated in cut14-208 (Fig. 3C). A small amount of fibrous chromatin DNA, apparently pulled, extended along the elongated mitotic spindle. The main portion of the nuclear chromatin, which still remained in the center of cells, also appeared to have been pulled, as both sides were conical. This phenotype was distinct from that of cut13-131. At least a portion of the chromosomes was separated and the spindle fully elongated in cut14-208. This phenotype resembles those of top2 (Uemura et al., 1986, 1987) and cut1 mutants (Hirano et al., 1986; Uzawa et al., 1990). In cut1 cells, however, much larger amounts of chromatin DNA were separated, displaying the characteristic archery-bow phenotype (Fig. 1). The phenotype of top2 mutant cells is very similar to that of cut14-208. In top2 mutants, the chromatin DNA pulled by the spindle was recently demonstrated to contain the centromere DNA, by
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fluorescence in situ hybridization (FISH). In order to examine the functional relationship between cut14+ and top2+ genes, we tested whether cut14-208 could be suppressed by a multicopy plasmid carrying the top2+ gene; however, no ts+ transformant cells were obtained by transformation. The double mutants cut14-top2 and cut14-top1 showed the cut phenotype at 36°C.

Characterization of cut15-85

The phenotype of cut15-85 was similar to that of cut14-208. Cells with the cut phenotype (open circles) and septate cells (filled squares) increased to the levels of 56% and 25%, respectively, at 36°C after 4 h (Fig. 4A). Cells displaying pulled nuclear chromatin were observed. The frequency of such aberrant mitotic cells made a peak (20%) at 2 h, but only 2% of the total cells showed such figures after 1 or 4 h. This pulled chromatin phenotype is similar, but not identical, to that of cut14-208. The overall structure of the nuclear chromatin was quite variable, often highly extended and deformed (Fig. 4B). In cut14-208, however, only a small portion of chromatin DNA was pulled. The amount of extended chromatin in cut15-85 was larger than that of cut14-208, and intermediate between top2 and cut1 mutants.

Characterization of other cut mutants

In cut16-267 mutants at 36°C, small portions of cells showed the nuclear chromatin stretched by the spindle (data
not shown). The frequency of cut17-275 cells with the cut phenotype was lower than that of septate cells (Fig. 5A); 66% of cells were septate, while 18% displayed the cut phenotype after 4 h at 36°C. DAPI stained cut17-275 cells after 2 h at 36°C (Fig. 6A) showed interphase chromatin in one side of the septate cells and no nucleus in the other, and the nuclear chromatin was pulled. The extended chromatin was often asymmetrical, extending in only one direction, along the spindle axis. In addition to the septate cells, cells without a nucleus were frequently seen. Such anucleate cells might be produced by cytokinesis of septate cells with a single nucleus.

The frequency of the cut phenotype in cut18-447 (Fig. 5B) was approximately 40% after 4 h at 36°C. Cells with stretched or condensed nuclear chromatin were infrequently noted prior to cytokinesis. Cells incubated at 36°C for 3 h showed DAPI-stained nuclear chromatin that was mostly of the interphase type. Nuclear chromatin in mitotic cells, however, was highly variable in morphology (Fig. 6B). Stretched nuclear chromatin very similar to that found in
cut1 or cut2 was seen. Seven per cent of the cells had condensed chromosomes, usually clustered, after 3 h.

Cells with condensed chromosomes were also observed in cut19-708 and two sterile strains. Strains 523 and 666 displayed such chromosomes in 14% and 17% of cells, respectively, after 2-3 h at 36°C. The frequency of the septate cells was high, 45%-60%, after 4 h at 36°C, while cells showing the cut phenotype were less frequent, 14%-22%. 523 was auxotrophic (poor growth in EMM medium containing leucine), but the segregation of ts and auxotrophy did not complement the ts phenotype of 666.

**DISCUSSION**

Fission yeast ts mutants were previously screened for those exhibiting the cut phenotype at the restrictive temperature (Hirano et al., 1986). After examining 587 ts strains, 18 were obtained which made up 10 complementation groups, including top2. In the present study, a new collection of 717 ts mutants (Matsumoto and Beach, 1991) was screened, and 25 cut mutants, making up 12 complementation groups, were isolated. Five genetic loci, top2, cut1, cut5, cut7 and cut9, were identified in the previous and the present cut mutant isolation. Thus, a total of 17 genetic loci producing the cut phenotype were obtained from approx. 1300 ts strains, making up roughly 1.3% of the ts strains examined. Because 12 of the loci consist of only one allele, the saturation point for cut mutants has not yet been reached, and many more cut mutants may be isolated. The S. pombe genome consists of approximately 1.4x10^7 bp (Fan et al., 1988), probably containing 7,000-10,000 genes. Assuming that 30% of the genes can generate ts mutants, approximately 25-40 loci may be expected to produce the ts cut phenotype. This number is not surprisingly large, considering the complexity of cell division mechanisms. cut mutants were also isolated from a collection of cold-sensitive (cs) mutants (Uemura et al., 1987).

Among 982 cs mutants cytologically examined at the restrictive temperature, only three showed the cs cut phenotype, and one was top2 defective. The reason for the low occurrence of cs cut mutants is not yet known. Higher temperature or higher growth rate may facilitate mitotic uncoupling in ts mutant cells, which results in the cut phenotype. Alternatively, the products of many cut+ genes might be enzymes, so that denaturation of mutant enzymes at low temperature might occur only infrequently. cs mutations may frequently be found in genes, whose products are needed for making higher-order structures by protein-protein interactions.

The cut+ gene products so far identified are cut1+ and cut2+ (Uzawa et al., 1990), cut7+ (Hagan and Yanagida, 1990), cut8+ and cut9+ (I. Samejima and M. Yanagida, unpublished, quoted by Goebel and Yanagida, 1991) in addition to top2+ (e.g. see Shiozaki and Yanagida, 1991, 1992). Among those, only two gene products (top2+ and cut7+) are understood in terms of molecular functions in mitotic chromosome disjunction (e.g. chromosomal DNA topology alteration and kinesin-related motor activity for spindle formation). cut1+ and cut2+ genes are possibly involved in the regulation of spindle pole body duplication (Uzawa et al., 1990); the cut1 protein is large (210 kDa), containing a putative nucleotide binding sequence and a COOH domain similar to that of the budding yeast ESP1.

The cut9+ gene product is similar to that of the budding yeast CDC16 gene (Icho and Wickner, 1987), containing the TPR (tricopeptide repeat) repeats (Goebel and Yanagida, 1991), and appears to be required for spindle elongation in the metaphase/anaphase progression (I. Samejima and M. Yanagida, unpublished result).

Nine strains among the 25 ts cut mutants isolated in the present study were allocated to seven new genetic loci (designated cut13 to cut19). Their cytological phenotypes have been classified into three groups. One is similar to that of top2 or cut1 mutants, displaying a portion of nuclear chromatin pulled by the spindle. In a top2 mutant most of the chromosomal DNA remained unseparated, while in a cut1 mutant a large part of the DNA was temporarily separated. The cytological phenotype of cut14-208 cells resembled that of top2, while that of cut15-85 was similar to that of cut1. cut16 and cut17 mutant cells also displayed...
stretched chromosomes. Another type of mutant showed condensed chromosomes before abnormal cell separation. Condensed chromosomes were seen in cut7 and cut9 mutants with half-spindles and a short spindle, respectively. cut13-131 belongs to this group, displaying a metaphase-like short spindle or somewhat elongated spindle with non-separated condensed chromosomes. The cytological phenotypes of the remaining cut16 and cut19 mutants were not well characterized. In cut18, cells revealed stretched chromatin as well as condensed chromosomes. Judging from the heterogeneous nature of the gene products and the variability of the phenotypes, the cut gene products appear to be involved in various aspects of cell division. A common characteristic of the mutant phenotypes is that the loss of any cut genes causes abnormal cell separation, uncoupled from nuclear division. We are currently engaged in identifying their gene products and investigating their participation in mitosis.

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