A Gene Required for the Separation of Chromosomes on the Spindle Apparatus in Yeast

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Summary

We describe the phenotypes caused by a cold-sensitive lethal mutation (ndc1-1) that defines the NDC1 gene of yeast. Incubation of ndc1-1 at a nonpermissive temperature causes failure of chromosome separation in mitosis but does not block the cell cycle. This defect results in an asymmetric cell division in which one daughter cell doubles in ploidy and the other inherits no chromosomes. The spindle poles are properly segregated to the two daughter cells. The primary visible defect is that the chromosomes remain associated with only one pole, and are thus delivered to one daughter cell. Meiosis II, but not meiosis I, is sensitive to the ndc1-1 defect, suggesting that NDC1 is required for some feature common to mitosis and meiosis II. ndc1-1 appears to define a new class of cell cycle gene required for the attachment of chromosomes to the spindle pole.

Introduction

The genetics of the yeast mitotic cell division cycle have been studied extensively (for review see Pringle and Hartwell, 1981). Mutations that affect a single step in the progress of the cell cycle have been recognized as conditional-lethal mutations that cause cells to arrest as a morphologically homogeneous population after shift to restrictive conditions (Hartwell et al., 1970). Over 50 cell-division-cycle (cdc) genes have been identified by such mutations. Among this large collection of mutations, though, very few have been demonstrated to have a direct effect on the function of the spindle apparatus.

Recently, conditional-lethal mutations in the yeast gene encoding beta-tubulin have been isolated (Neff et al., 1983; Thomas et al., 1985) and shown to result in the arrest of the cell cycle at mitosis (J. H. Thomas, Ph.D. thesis, Massachusetts Institute of Technology, 1984; T. Huffaker, personal communication). Drugs such as methyl benz-imidazole-2-yl-carbamate (MBC), which lead to the depolymerization of microtubules, cause a similar cell cycle arrest (Wood and Hartwell, 1982). This observation suggests that mutations causing a complete defect in spindle function should have a classical Cdc phenotype. In principle, however, mutations that affect the process of chromosome segregation in other ways, such as preventing attachment of the spindle to chromosomes, might well behave differently.

We describe a cold-sensitive lethal mutation, ndc1-1, that causes a cell cycle specific defect in chromosome segregation, but does not result in a classical Cdc arrest. We present evidence that ndc1-1 causes failure of the chromosomes to attach to one pole of the spindle during the mitotic cell cycle, and in the second (but rarely the first) meiotic division.

Results

Isolation of the ndc1-1 Mutation

The strain J76, carrying the ndc1-1 mutation, was obtained by Moir et al. (1982) by screening for cdc mutations. We reexamined strain J76 because its terminal arrest phenotype appeared superficially similar to that of beta-tubulin cold-sensitive mutants (Thomas, Ph.D. thesis, 1984). We recovered the ndc1-1 mutation from strain J76 as described in detail in Experimental Procedures. Briefly, strain J76 appeared to be diploid or polyploid, but by extensive backcrossing to wild-type haploid strains we isolated a haploid derivative of J76 that carried a single mutation responsible for its cold sensitivity. We named the gene defined by this mutation NDC1 (nuclear-division-cycle), since the arrest phenotype of the mutant suggested a defect in nuclear division (see below). The cold-sensitive mutation derived from J76 was named ndc1-1. We genetically mapped the ndc1-1 gene on chromosome 13 to a region to which no identified cdc gene maps (see Experimental Procedures). We conclude that ndc1-1 defines a new cell cycle related gene in yeast.

Production of "Aploid" Cells by ndc1-1

To determine the nonpermissive arrest phenotype of ndc1-1, asynchronous cultures of a wild-type strain or a strain carrying ndc1-1 were grown at 30°C (permissive temperature) and shifted to 13°C (nonpermissive temperature). At time points after shift, cell types and the position and appearance of the nuclear DNA were determined. The results of one such experiment are shown in Table 1. In the ndc1-1 strain, large-budded cells accumulated to about 65% of the total, peaking at about 2 generation times after shift to nonpermissive temperature. Subsequently this number dropped and the fraction of unbudded cells accumulated slowly for the next several generations. The morphology of the unbudded cells that accumulated was striking: they contained no detectable nuclear DNA, although they contained mitochondrial DNA that stained normally. After 3 generation times at nonpermissive temperature about 40% of the cells in the culture were unbudded, with no visible nuclear DNA stain. We call these cells "aploid." Since we never observed a budded aploid cell and the fraction of cells that were nucleated decreased steadily over the time course of arrest, we supposed that the nucleated cells must bud repeatedly (i.e. go through additional cell cycles), producing aploid daughters.

The simplest hypothesis to explain the generation of aploid cells was asymmetric segregation of the nucleus (or the nuclear DNA), giving rise to a diploid and an aploid daughter. We tested two predictions of this hypothesis:

Table 1. Percentage of Various Cell Types at Times after Shift of Wild-type or *ndc1-1* Cells to Nonpermissive Temperature

Strain	Generation Times after Shift	% Unbudded Cells with Nuclear DNA	% Unbudded Cells without Nuclear DNA	% Small-Budded Cells with Nuclear DNA	% Large-Budded Cells with 1 Region Nuclear DNA	% Large-Budded Cells with 2 Regions Nuclear DNA
Wild-type	2.0	60	0	23	----------- 17[a]	-----------
ndc1-1	0	64	0	26	----------- 10[a]	-----------
ndc1-1	0.7	58	0	22	10	10
ndc1-1	1.5	21.5	0.5	20	51	7
ndc1-1	2.6	10	22	7	59	2
ndc1-1	3.3	5	40	8	47	0

Wild-type (DBY1398) or *ndc1-1* (DBY1503 *MATa his4-539 ade2 ura3-52 ndc1-1*) cells were shifted from 30°C to 13°C at time 0. At time points after shift cells were harvested, fixed, and stained, and the cell and nuclear morphology scored as described in Experimental Procedures. At least 200 cells were scored for each sample. The generation time of wild-type cells at 13°C is 8 to 10 hr.

[a] The great majority of large-budded wild-type cells (and *ndc1-1* cells at 30°C) contain two regons of nuclear DNA; however, the fraction depends strongly on what is scored as a large-budded cell.

first, the production of a diploid daughter from a haploid parent cell; second, the continuation (in the nucleated daughter) of the cell cycle through additional rounds of budding despite the defect in nuclear division.

Doubling of Ploidy by *ndc1-1*

To test the first prediction, haploid *ndc1-1* cells grown at 30°C were shifted to 13°C for 18 hr (about 1.5 generation times). At this time most of the cells had accumulated as large-budded doublets, nearly all of which had nuclear DNA in only one cell body (Table 1). The large-budded doublet cells were micromanipulated to prerecorded positions on solid medium and incubated at 30°C. For two generations these cells were inspected and when they divided the daughters were separated by micromanipulation to new positions. If the daughters grew into colonies at 30°C, their ploidy was determined (see Experimental Procedures). A summary of the cell lineage and resulting ploidy predicted by our hypothesis is shown in Figure 1.

When a wild-type haploid strain was subjected to this regime, the daughter cells were all viable and haploid (10/10 complete lineages). However, when an *ndc1-1* strain was used, 19 of 20 lineages were clearly different from the wild-type. The mutant lineages fell into three classes:

Class 1: eight cells gave rise to one daughter that was viable and diploid and one daughter that never budded. These precisely conform to the prediction of our hypothesis (Figure 1).

Class 2: seven cells gave rise to two inviable daughters. In each case, one of the daughters never budded, but the other budded and divided once or twice. The source of this inviability is not known, but could be due to chromosome loss to the aploid daughter (never budded) during an otherwise typical (class 1) *ndc1-1* division.

Class 3: four cells gave rise to two daughters, one of which produced two viable diploid daughters, the other of which variably produced dead cells and diploids. The source of this minority class is also unclear, but note that diploidization has resulted in these cells as well as in class 1 cells.

In many of the cells (and in two-thirds of the viable cells) the prediction that diploidization of one daughter would correlate with the death of the other (presumed aploid)

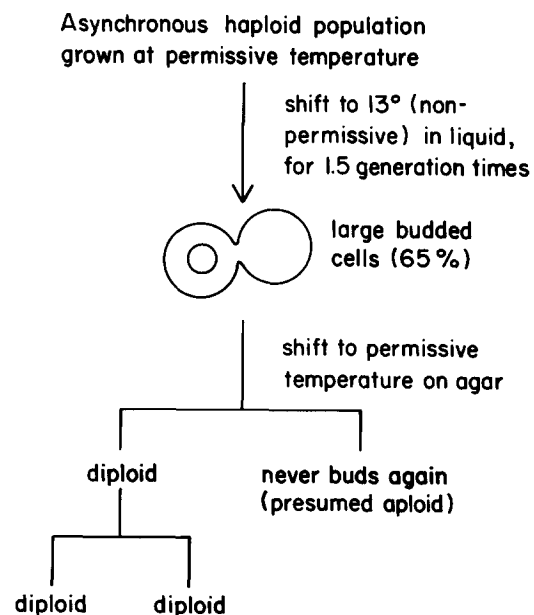Asynchronous haploid population grown at permissive temperature

Figure 1. The Inferred Pattern of Diploidization of *ndc1-1* Cells upon Shift to Restrictive Temperature

The diagram shows the result most often observed after shift of *ndc1-1* cells (DBY1583 *MATa his4-539 ura3-52 lys2-801 ndc1-1*) transiently to nonpermissive temperature as described in the text. Wild type (DBY1398 *MATa ade2 ura3-52*) invariably produced four viable haploid cells.

daughter was confirmed. If the lethality of cells in class 2 is indeed due to chromosome loss, this places an upper limit on the frequency of chromosome pairs segregating to the "aploid" cell of about 1 out of 25 (yeast has at least 16 chromosomes).

ndc1-1 Does Not Cause a Classical Cell Cycle Arrest

The second prediction of the hypothesis of asymmetric nuclear division is that the nucleated (diploid) daughter should continue to progress through the cell cycle, producing additional aploid cells. To test this prediction we followed the progress of the cell cycle of *ndc1-1* cells
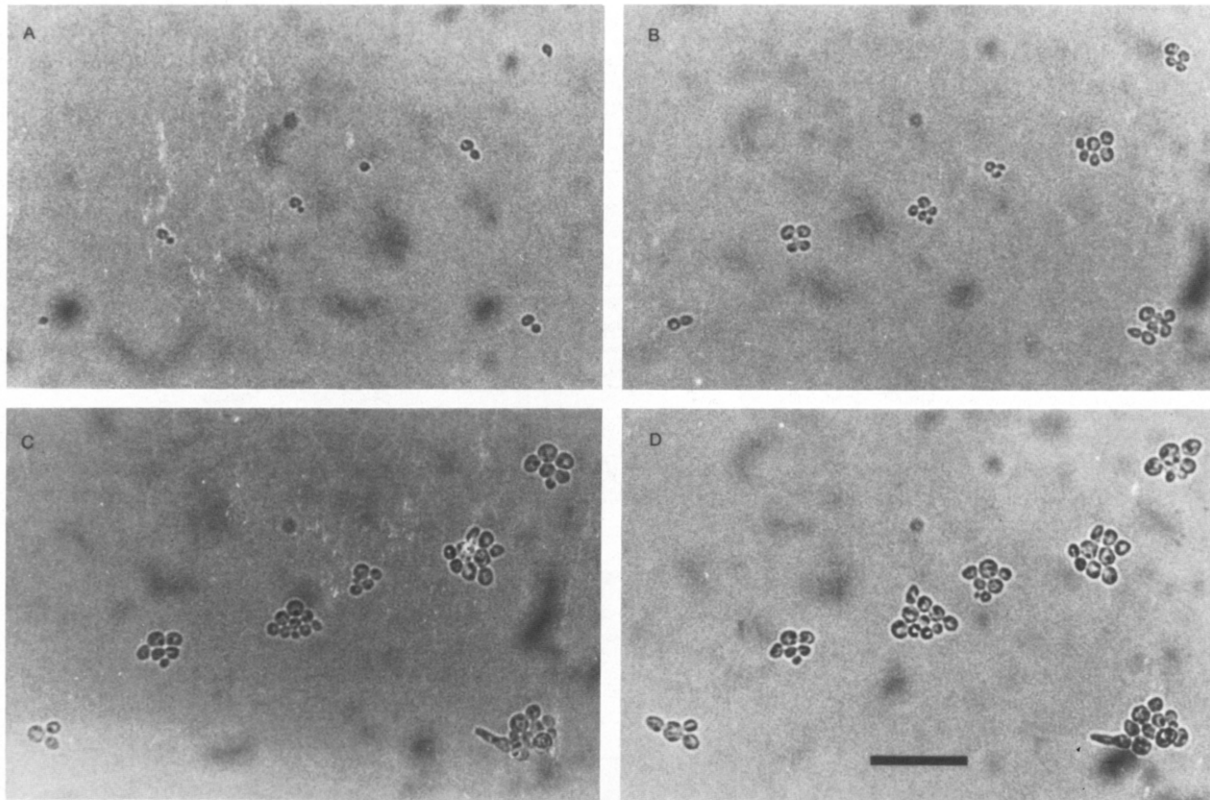
Figure 2. Time Lapse Photomicroscopy of *ndc1-1* Cells Growing at Nonpermissive Temperature on Solid Medium

ndc1-1 cells (DBY1583) mounted on an agar slab were observed over a time course at 11°C (nonpermissive temperature). At the following times after shift, a photograph was taken of the same field of cells: (A) 0 hr, (B) 24 hr, (C) 53 hr, (D) 78 hr. The generation time of wild-type strains at this temperature is about 12 hr. Bar: 20 microns.

shifted to fully nonpermissive temperature (11°C) on solid medium prepared on microscope slides (see Experimental Procedures); this technique allows the observation of the same cell over time (Hartwell et al., 1970). Wild-type cells at 11°C and *ndc1-1* cells at permissive temperature grew with a normal doubling time on the slides and appeared morphologically normal.

Photographs of typical *ndc1-1* cells over a time course at 11°C are shown in Figure 2. Data accumulated from a larger number of cells are shown in Table 2. An average of 6.3 daughters was produced by each cell over about 5 generation times; thereafter production of new daughter cells declined sharply and we began to observe cell lysis. We conclude that, under conditions fully nonpermissive for the nuclear division phenotype of *ndc1-1*, the major morphological events of the cell cycle are not blocked for at least three generations. We speculate that the ploidy of the nucleated cell doubles at each division.

The *ndc1-1* Defect Is Cell Cycle Specific

In the previous experiment, we noted that cells that were large-budded at the time of the temperature shift gave rise on the average to twice as many daughters as unbudded cells (Table 2). This fact suggests that the large-budded cells have passed the execution point for a cell cycle spe-

Table 2. Mean Number of Cell Bodies at Times after Shift of *ndc1-1* to Nonpermissive Temperature

Cell Morphology at Time of Shift	1.5 Generation Times	3.3 Generation Times	4.4 Generation Times
Unbudded	2.7	4.1	4.6
Small-budded	4.2	6.2	7.5
Large-budded	5.5	8.1	9.0

ndc1-1 cells grown at 30°C (permissive temperature) were sonicated lightly to separate cells and plated on agar slabs at 11°C (nonpermissive temperature). Individual cells were followed throughout the arrest by photomicroscopy of the same fields at each time point (see Experimental Procedures). At least 20 cells of each initial morphology (unbudded, small-budded, and large-budded) were observed.

The mean number of cell bodies is the apparent number of cells as determined from observation of the cell outlines, without attempting to determine whether or not they are physically separate.

cific defect that is imposed in the *ndc1-1* mutant, and can therefore divide once normally.

We tested directly whether expression of the diploidization phenotype of *ndc1-1* requires progression through the cell cycle. An asynchronous culture of haploid *ndc1-1* cells (DBY1583) or *NDC1* cells (DBY1579, *MATa his4*) were grown at 30°C and shifted to nonpermissive temperature
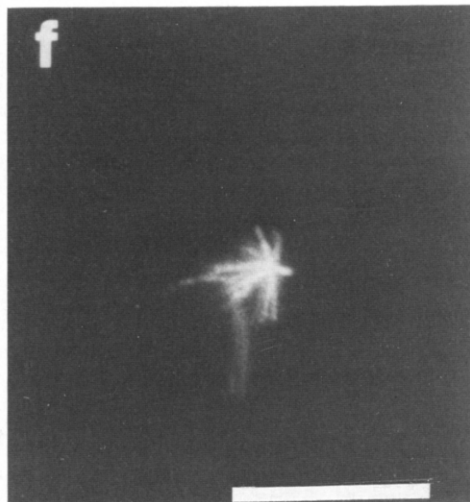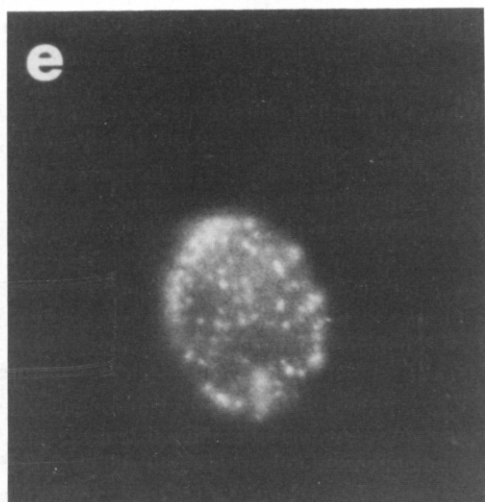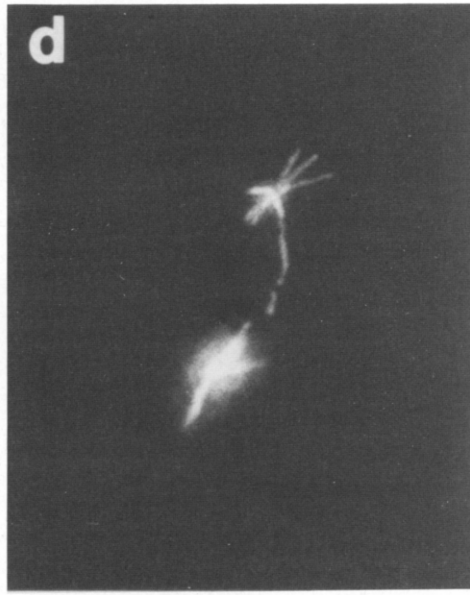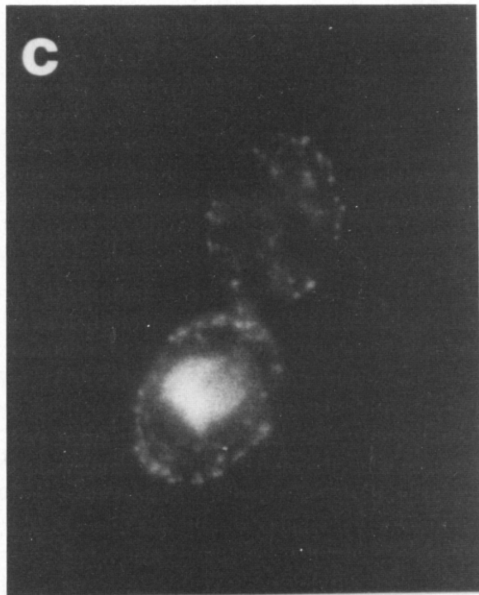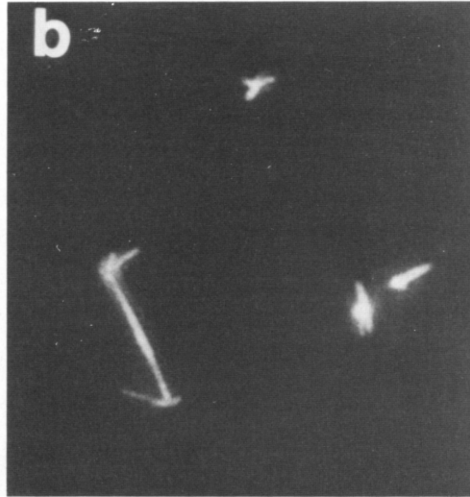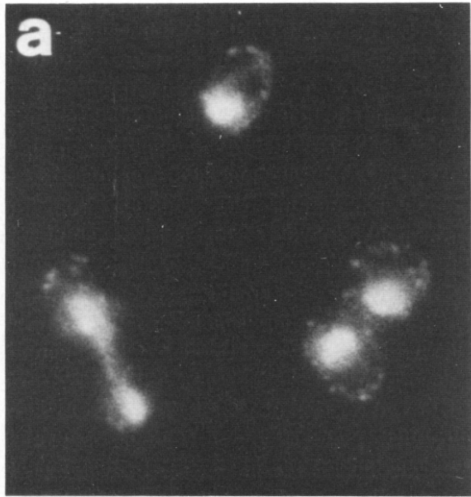
(11°C) in the presence of either alpha-factor or hydroxy-urea (HU). Alpha-factor causes arrest of the cell cycle in G1 (Bucking-Throm et al., 1973), and HU causes cell cycle arrest at DNA synthesis (Hartwell, 1976). After incubation for 20–24 hr to allow accumulation at the HU or alpha-factor block, the cells were simultaneously shifted to 30°C and out of drug, and micromanipulated to agar plates, where they were allowed to form colonies at 30°C and their ploidy was determined (see Experimental Procedures).

If diploidization does not require progression through the cell cycle, then nearly all of the viable cells should become diploid, as they do in the absence of a cell cycle block. If diploidization requires progression through the cell cycle, then some fraction of the cells should remain haploid. Assuming ndc1-1 acts at only one point in the cell cycle, the fraction of haploid cells should reflect the point in the cell cycle at which the ndc1-1 defect is expressed (the execution point, Hartwell, 1971). In the alpha-factor block, 84% (38/45) of the ndc1-1 cells remained haploid, the remainder had become diploid. When HU was used, about 75% (12/16) of the ndc1-1 cells remained haploid, the remainder had diploidized. Control experiments showed that the ndc1-1 cells before shift were all haploid, and that, under identical regimes, NDC1 (wild-type) cells all remained haploid. These data are consistent with the simple idea that there is a single execution point for the ndc1-1 defect and that this execution point is about 0.3 of the cell cycle after "start" (two times the fraction of diploidized cells from the alpha-factor block, since the cells that remain haploid divide once during the experiment).

ndc1-1 Cells Have a Spindle at Nonpermissive Temperature

Since ndc1-1 displays a defect in chromosome separation we wanted to determine whether it alters spindle structure, as assayed by direct immunofluorescence using antibodies directed against tubulin. Representative stages of the cell cycle are shown in the wild-type cells in Figure 3 (for review see Byers, 1981). All microtubules in yeast appear to have one end associated with the spindle pole body (or bodies), which remains embedded in the nuclear envelope throughout the cell cycle (in yeast the nuclear envelope remains intact during mitosis). Some microtubules

radiate into the nucleus to form the spindle, others radiate outward to form cytoplasmic microtubules. In the course of the cell cycle the single spindle pole body duplicates and the two products migrate to opposite sides of the nuclear envelope, where the spindle forms between them. After completion of DNA synthesis, the nucleus migrates into the bud neck and the spindle and nucleus elongate through the bud neck. This serves to segregate the chromosomes, closely associated with each of the spindle pole bodies, to each cell. In wild-type cells the position of the spindle pole body or bodies can be inferred using immunofluorescence as the vertex at which different bundles of microtubules converge (see Figure 3).

To determine the pattern of microtubules in ndc1-1 cells we used a monoclonal antibody (YOL1/34) to yeast tubulin (Kilmartin et al., 1982) and a secondary antibody conjugated to rhodamine as an immunofluorescent probe. NDC1 and ndc1-1 cells grown asynchronously at 30°C were shifted to 13°C and time points were prepared for immunofluorescence and DAPI stain (Experimental Procedures). The patterns of microtubules seen in ndc1-1 cells grown at 30°C and wild-type cells before and after shift to 13°C were indistinguishable (not shown).

The microtubules in ndc1-1 cells at 13°C remained intact and appeared normal prior to mitosis. Late in the cell cycle, after completing mitosis at the nonpermissive temperature, the microtubules in ndc1-1 cells also appeared similar to wild-type: bundles of microtubules radiated from two distinct points (presumed to be spindle pole bodies) in the large-budded cells, one in each cell body (compare Figures 3b and 3d). ndc1-1 cells differed from wild-type in that there was visible nuclear DNA associated with only one of the two clusters of microtubules. The segregation of the putative spindle pole bodies to the two daughter cells occurred with high fidelity in ndc1-1 cells despite the lack of chromosome separation. We have not observed elongated spindles in ndc1-1 cells undergoing mitosis at 13°C, suggesting that at the time of mitosis ndc1-1 spindles differ in some way from wild-type spindles.

The Aploid Cell Is Equally Likely to be Mother or Daughter

Yeast mitosis is intrinsically asymmetric. Early in the cell cycle the nucleus resides exclusively in the mother cell,

Figure 3. Wild-type and ndc1-1 Cells Incubated at Restrictive Temperature and Fluorescently Stained for DNA and Tubulin

A wild-type (DBY1398) or ndc1-1 strain (DBY1583) was grown to mid-logarithmic phase at 30°C and shifted to 13°C in liquid medium. After about 1.5 generation times (16 hr) cells were processed for immunofluorescence as described in Experimental Procedures. Each pair of photographs shows a cell stained with DAPI (left) or with the antitubulin antibody YOL/34 (right). Cell shape can be discerned by the pattern of punctate DAPI staining of the mitochondrial DNA.

(a and b) Typical wild-type cells in various stages of the cell cycle. The top cell is unbudded, with unseparated spindle pole bodies and associated microtubules. The lower left cell is large-budded, with an elongated spindle and chromosomal DNA just completing segregation. The lower right cell has completed nuclear and spindle division. Indistinguishable patterns are observed in wild-type cells after shift to 13°C and in ndc1-1 cells grown at 30°C (not shown).

(c–f) Typical ndc1-1 cells. Note that the cells are considerably larger than wild-type, presumably because their cell cycle is considerably slowed but cell growth continues.

(c and d) Typical large-budded cell with unseparated nuclear DNA but with apparently normally duplicated and segregated spindle pole bodies with associated microtubules. This pattern is quite similar to that of normal cells just after nuclear division (compare with b), except that the nuclear DNA is undivided.

(e and f) Typical aploid cell, overexposed for DAPI stain to show the lack of detectable nuclear DNA. The cell appears to contain a spindle pole body with normal associated microtubules, although the array of microtubules is more extensive than in the typical wild-type unbudded cell. Bar: 5 microns.

Table 3. Types of Asci Produced at Various Temperatures by *ndc1-1* and *NDC1* Strains

Strain	Relevant Genotype	Sporulation Temperature	Cell Morphology (%)		
			3- or 4-Spored Asci	2-Spored Asci	Unsporulated
DBY1401 × DBY1396	*NDC1/NDC1*	26°C	42	13	44
		17°C	56	5	39
		14°C	57	4	39
		11°C	33	4	63
DBY1674 × DBY1671	*ndc1-1/NDC1*	26°C	37	11	52
		14°C	56	6	38
		11°C	48	2	50
DBY1674 × DBY1670	*ndc1-1/ndc1-1*	26°C	43	13	44
		14°C	24	24	52
		11°C	6	17	77
DBY1582 × DBY1583	*ndc1-1/ndc1-1*	26°C	48	19	33
		17°C	54	7	39
		14°C	30	19	51
		11°C	9	27	64

Diploid parent strains to be sporulated were grown mitotically at 26°C and shifted to the indicated temperature simultaneously with shift to conditions that induce sporulation, as described in Experimental Procedures. In each case 200 asci were counted, except DBY1401 × DBY1396 (26° and 17°C) and DBY1582 × DBY1583 (26° and 17°C), for which 100 asci were counted. Strains: DBY1401 *MATα ade2 lys2-801*, DBY1396 *MATa his4-539 lys2-801*, DBY1670 *MATa trp1-1 ura3-52 lys2 ndc1-1*, DBY1671 *MATa trp1-1 ura3-52 lys2*, DBY1674 *MATα ade6 leu1 cyh2 lys2 can1 ndc1-1*, DBY1582 *MATα lys2-801 ade2 ndc1-1*.

separated from the growing bud by a very narrow cytoplasmic neck (narrower than the nuclear diameter, Byers, 1981). The nucleus and chromosomes thus require active movements to pass through the bud neck, and it seemed possible that the chromosomes, during *ndc1-1* arrest, remain together because no active force is generated to separate them. If so, the chromosomes should remain in the mother cell.

To determine where the nuclear DNA ended up during an *ndc1-1* arrest, the mother cell was "marked" by preincubation with alpha-factor. This treatment leaves the mother cell in a permanent "shmoo" shape (oblong with a projection at one end). The bud produced by this shmoo (after release from the alpha-factor arrest) is the normal round shape and can be distinguished from the shmoo-shaped mother cell. *ndc1-1* cells (DBY1583) were arrested with alpha-factor for 3 hr at 26°C, at which time 80% of the cells were shmoo-shaped. The cells were then simultaneously removed from alpha-factor and shifted to 13°C. At time points, large-budded cells were scored for the position of their nuclear DNA. After 0.7 generation times, the nuclear DNA usually resided in the mother cell, but sometimes in the daughter cell or in the bud neck, presumably in transit between them (58 : 15 : 9, DNA in the mother cell : daughter cell : bud neck). After 1.0 or more generation times the nuclear DNA appeared randomly in the mother and daughter cells (45 : 43 : 22, mother : daughter : neck). To confirm this result, aploid daughter cells were scored for being shmoo-shaped or not. These counts indicated that both mother and daughter cells could become aploid (38 shmooed : 57 nonshmooed, at 1.4 generation times or later). Since only 80% of the mother cells were shmoo-shaped, this result indicates that the chromosomes end up about 50% of the time in each cell type.

Since the alpha-factor treatment could have affected the outcome of the previous experiment, we wanted to confirm this result using a technique that did not perturb the parent cells. We took advantage of the fact that mothers (but not daughters) have chitin rings (called bud scars) that can be visualized by fluorescence microscopy using Calcafluor (Cabib, 1975). We stained *ndc1-1* cells for nuclear DNA and with Calcafluor after incubation at 13°C for 2 generation times. Of 29 large-budded cells scored, 16 had nuclear DNA in the mother, 11 in the daughter, and 2 in the neck. This result is not quantitatively reliable, since high fluorescent background prevented scoring of most cells. Nonetheless this result lends support to the previous experiment and suggests that alpha-factor prearrest did not affect its outcome.

The Effect of *ndc1-1* on Meiosis

Given its phenotype in mitotic cells, it was of interest to determine the effect of *ndc1-1* on meiotic chromosome segregation. In order to test the effect of *ndc1-1* on meiosis we constructed diploids homozygous or heterozygous for *ndc1-1* and compared their sporulation to that of a wildtype diploid. The diploids were grown at 30°C, then shifted to temperatures ranging from 30°C to 11°C simultaneously with shift to medium that induced sporulation, and the types of asci produced were quantitated. Yeast strains can generate asci containing from one to four spores. Although *ndc1-1* cells produced asci at all temperatures, the *ndc1-1* homozygote produced an abnormally high frequency of two-spored asci when sporulated at 14°C or 11°C (Table 3). Wild-type strains and strains heterozygous for *ndc1-1* produced about the same high fraction of three- or four-spored asci at all temperatures from 11°C to 30°C (80%–90% of all asci, Table 3). Homozygous *ndc1-1*
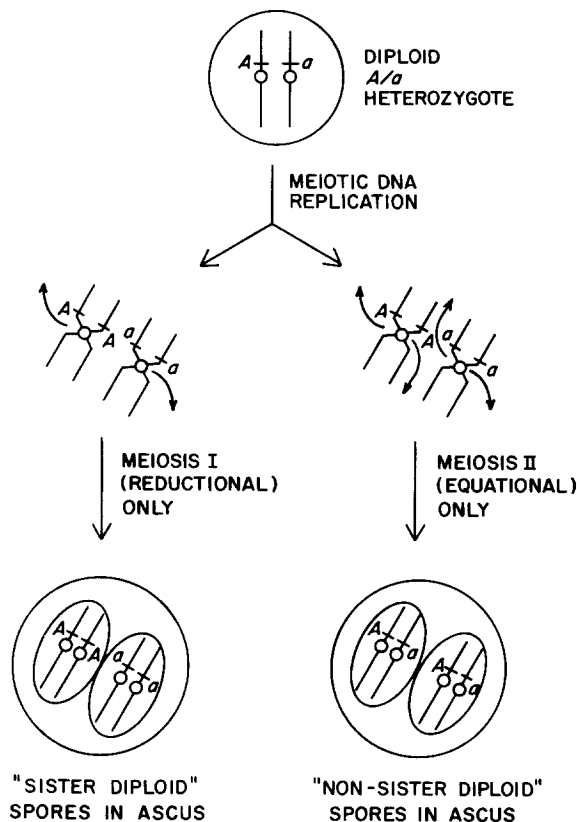
Figure 4. Schematic Diagram of Aberrant Chromosome Segregation Patterns That Give Rise to Two-Spored Asci in *ndc1-1* Meiosis

A mitotically growing diploid, heterozygous for a tightly centromere linked marker (A/a), undergoes premeiotic DNA replication normally. On the left is shown meiosis of the pattern 2 type (see text), in which only the meiosis I chromosome segregation occurs. The two diploid nuclei that result are packaged into two spores, each of which is diploid and carries sister chromatids (A/A or a/a, a "sister diploid" spore). On the right is shown meiosis of the pattern 3 type (see text), in which only the meiosis II chromosome segregation occurs. The two diploid nuclei that result are packaged into two spores, but in this case each spore carries nonsister chromatids (A/a, a "nonsister diploid" spore).

diploids produced a progressively larger proportion of two-spored asci as the temperature of sporulation decreased, although even at 11°C not all asci were two-spored, suggesting that *ndc1-1* imposes an incomplete or leaky defect in meiosis.

Most Two-Spored Asci Caused by *ndc1-1* Arise from Failure in Both Meiosis II Chromosome Separations

Viable two-spored asci could arise (simply) from three different defective patterns of meiosis, each of which results in a different pattern of genetic markers in the two spores:

—Pattern 1: the two spores arose from a normal meiosis but only two nuclei were packaged into spores (this is the source of two-spored asci in wild-type, see below and Davidow et al., 1980). In this case both spores will be haploid.

—Pattern 2: meiosis I was normal, but both meiosis II chromosome separations failed to occur, and the resulting

diploid genomes were each packaged into a single spore. In this case both spores will be diploid and will carry sister chromatids for each of the chromosomes. We call these "sister diploid" spores (see Figure 4).

—Pattern 3: the meiosis I chromosome separation failed, a normal meiosis II followed, and the resulting diploid genomes were each packaged into a single spore. In this case both spores will be diploid, and will carry nonsister chromatids for each of the chromosomes. We call these "nonsister diploid" spores (see Figure 4).

To distinguish these patterns of meiosis, the diploid strains tested were heterozygous for a number of markers. Three markers were used to distinguish haploid from diploid spores: *MAT*, *can1*, and *cyh2*, each of which can be scored for heterozygosity in a plate test (Experimental Procedures). Since each of these markers recombines freely with its centromere, each is expected to be heterozygous in about two-thirds of all diploid spores; together they constitute a reliable indicator for diploidy. Three other recessive heterozygous markers were used to distinguish sister and nonsister diploid spores: *leu2*, *trp1*, and *ura3* (each linked to a different centromere). Since these markers are linked to their respective centromeres, they mark sister chromatids in meiosis. Thus two sister diploid spores from a two-spored ascus will be unlike in phenotype (one positive and one negative), while both nonsister diploid spores will be heterozygous for all three markers (and hence phenotypically positive). The reliability of these methods was established by sporulating examples of sister and nonsister diploids at permissive temperature and testing their genotypes directly by tetrad analysis.

Using these tests we determined the genetic composition of the spores in two-spored asci from wild-type sporulated at 30°C and from *ndc1-1* homozygous diploids that had been sporulated at 30°, 14°, and 11°C. As expected, the two-spored asci produced by wild-type and *ndc1-1* at 30°C were from a normal meiosis in which only two nuclei were packaged into spores (Table 5). The segregation of markers in the *ndc1-1* homozygote sporulated at 11° or 14°C indicated a different cause for their two-spored asci. The detailed data for three representative two-spored asci are shown in Table 4, and data for all asci are summarized in Table 5. Consider ascus 2 from Table 4 as an illustration of our analysis. Both spores were diploids, since they were heterozygous for both mating type and *cyh2*. Each diploid from ascus 2 carries sister chromosomes, as indicated by the fact that one spore was negative (homozygous since negative is recessive) and one positive for each of the centromere linked markers. Thus ascus 2 is the result of a proficient meiosis I followed by failure in meiosis II for both sets of chromosomes.

Table 5 shows the classification of all of the two-spored asci. At both 14° and 11°C the predominant event in *ndc1-1* homozygotes was failure of both meiosis II divisions to produce two sister diploid spores. However, at each temperature about 10% of the events appeared to be failure of meiosis I followed by a proficient meiosis II. Some two-spored asci were similar to those observed in wild-type, containing two haploid spores (this was expected because of the leakiness of the *ndc1-1* defect in meiosis).

Table 4. Examples of Marker Segregation in Two- and Three-Spored Asci of an *ndc1-1* Diploid Sporulated at 11°C or 14°C

Parental Genotype:	MATα	leu1	cyh2	+	can1	+	ndc1-1
	MATa	+	+	ura3	+	trp1	ndc1-1

		Spore Phenotype						
Ascus	Spore	MAT	Can	Cyh	Ura	Leu	Trp	Interpretation
1	A	a	S/R	S/R	+	−	+	Sister diploids
	B	α	S/R	S/R	−	+	−	
2	A	NM	R	S/R	+	+	−	Sister diploids
	B	NM	S	S/R	−	−	+	
3	A	NM	S/R	S/R	+	+	+	Nonsister diploids
	B	NM	S/R	S/R	+	+	+	
4	A	NM	S/R	S/R	+	+	−	Sister diploid
	B	a	R	R	−	−	+	Haploid
	C	α	S	S	−	−	+	Haploid
5	A	NM	R	S/R	+	−	+	Sister diploid
	B	a	S	S	−	+	−	Haploid
	C	α	S	R	−	+	−	Haploid
6	A	a	S	S	−	+	−	Haploid
	B	α	R	R	+	−	+	Haploid
	C	a	R	S	+	−	+	Haploid

The strain (DBY1670 × DBY1674) was sporulated as described in Table 3, at 11° or 14°C. Two-spored asci from the 11°C sporulation or three-spored asci from the 14°C sporulation were dissected as described in Experimental Procedures.

NM: nonmatter, indicating heterozygosity at the mating type locus. a: mating-type a. α: mating-type α. S: sensitive. R: resistant. S/R: heterozygous at the locus, indicated by a sensitive strain (S is dominant) that produces frequent resistant papillae by mitotic recombination, see Experimental Procedures. Can: 50 µg/ml canavanine. Cyh: 1 µg/ml cycloheximide.

Table 5. Types of Meiosis Events Giving Rise to Two-spored Asci for *NDC1* and *ndc1-1* Strains at Permissive and Nonpermissive Temperatures

Strain	Relevant Genotype	Sporulation Temperature	Inferred Meiotic Event and Type of Spores Produced	Fraction of Total
DBY1386 × DBY1383	NDC1/NDC1	30°C	Normal, haploids	10/10
DBY1582 × DBY1583	ndc1-1/ndc1-1	30°C	Normal, haploids	19/19
DBY1670 × DBY1674	ndc1-1/ndc1-1	14°C	M II failed, sister diploids	24/34[a]
			M I failed, nonsister diploids	3/34[a]
			Normal, haploids	7/34
DBY1670 × DBY1674	ndc1-1/ndc1-1	11°C	M II failed, sister diploids	24/30[a]
			M I failed, nonsister diploids	3/30[a]
			Normal, haploids	3/30

Data similar to that shown in Table 4 were used to infer the types of meiotic chromosome segregation as described in the text. Strains were grown and sporulated as described in Table 3. For the wild-type and *ndc1-1* strains sporulated at 30°C, at least 90% of the two-spored asci had two viable spores. For *ndc1-1* strains sporulated at 11° or 14°C about 75%–85% of the two-spored asci had two viable spores.

[a] In each of these cases five or six nonmating spores were resporulated at permissive temperature and subjected to tetrad analysis to confirm their genotype, as described in the text.

Mixed Meiosis II Divisions Give Rise to Three-Spored Asci in *ndc1-1*

Three-spored asci arise in wild-type sporulations by a mechanism similar to that for two-spored asci: meiosis is normal, but only three haploid genomes become packaged into spores (Davidow et al., 1980). However, in *ndc1-1* strains sporulated at 14°C there were unexpectedly high numbers of three-spored asci (data not shown). It seemed possible that these might have arisen by failure at only one of the two meiosis II divisions, which would presumably give rise to a three-spored ascus containing two haploid spores, and one "sister diploid" spore. Such an event would be interesting because it would indicate proficiency in one meiosis II division and failure in the other within a single cell. To determine if this was the case, three-spored asci produced by *ndc1-1* diploids sporulated at 14°C were dissected and analyzed by the methods described for two-spored asci. Examples of the patterns of

Table 6. Types of Meiotic Events Giving Rise to Three-spored Asci in *ndc1-1* at 14°C

Strain	Relevant Genotype	Inferred Meiotic Event and Types of Spores Produced	Fraction of Total
DBY1674 × DBY1670	*ndc1-1/ndc1-1*	Failed in one M II, one sister diploid, 2 haploids	27/34[a]
		Normal, 3 haploids	5/34
		3 diploids, premeiotic tetraploidization?[b]	2/34

Data similar to those shown in Table 4 were used to infer the pattern of meiotic chromosome segregation as described in the text.
[a] Five of these spores that were nonmaters were resporulated at permissive temperature and subjected to tetrad analysis to confirm their genotype, as described in the text.
[b] This class may have arisen from a normal meiosis by an *ndc1-1* diploid that had doubled its ploidy at 14°C (to tetraploid) prior to meiosis.

marker segregation in representative three-spored asci are presented in Table 4, and data for all of the asci are summarized in Table 6. Nearly all of the three-spored asci arose from a similar defect in meiotic chromosome segregation, namely a normal meiosis I followed by one normal meiosis II division and one complete failure. The viability of the spores from these asci was high (84% of the three-spored asci produced three viable spores), ruling out the occurrence of high levels of nondisjunction during meiosis.

Discussion

We have presented evidence that the mutation *ndc1-1* causes a cell cycle specific defect in chromosome separation. This defect results in the delivery of all or nearly all of the chromosomes to only one of the daughter cells, doubling its ploidy. Our conclusions depend on two major pieces of evidence: genetic characterization of the effects of incubation of *ndc1-1* cells at restrictive temperature, and the morphology of mutant cells, particularly their microtubules and nuclear DNA.

The Spindle Poles Separate and the Chromosomes Are Moved in *ndc1-1* Cells

Microtubules in wild-type yeast always have one end associated with a spindle pole body (Byers and Goetsch, 1973, 1975), radiating both into and out of the nucleus. This rule appears to hold for *ndc1-1* cells, since unbudded *ndc1-1* cells have a single "vertex" of microtubules and the appearance of a second vertex coincides temporally with spindle pole body separation in wild-type cells. Furthermore, the two vertices in the *ndc1-1* arrest segregate reliably to the two daughter cells. These observations suggest that the spindle pole body has duplicated and properly segregated in the *ndc1-1* cells.

The mother–daughter symmetry of chromosome movement during the *ndc1-1* arrest limits the interpretation of the defect conferred by *ndc1-1*. Since the bud neck of Saccharomyces cerevisiae is smaller than the diameter of the nucleus (Byers and Goetsch, 1975), it is clear that only active migration can drive the nucleus and all of its chromosomes through it. Since the block in chromosome sep-

aration in *ndc1-1* is complete but the orientation of chromosome movement random, we infer that some structure (perhaps the spindle) actively moves the chromosomes to one pole or the other, but that the orientation of movement is random.

The Action of *NDC1* Is Cell Cycle Specific

Despite the lack of a uniform terminal arrest morphology, our double shift experiments with alpha-factor and HU indicate that the *ndc1-1* defect is expressed in a cell cycle specific manner. It is reasonable to suppose that the *ndc1-1* defect that causes diploidization is expressed at a single stage in the cell cycle (its "execution" point, Hartwell, 1976). If so, we can estimate the *ndc1-1* execution point to be about 0.3 of the cell cycle past the alpha-factor block. This coincides with the time of mitosis in wild-type cells. Lethality in the diploid products of an *ndc1-1* division would result in an underestimate of this distance.

If one accepts the idea that there are genes specific to the cell division cycle whose mutant phenotypes do not include a uniform terminal morphology, then it seems useful to broaden the definition of cell cycle gene. We propose that the ability to define an execution point (or points) might be a more general and useful criterion. Holm et al. (1985), in analyzing the phenotype of temperature-sensitive alleles of the *top2* gene of yeast, which encodes DNA topoisomerase 2, have come to a similar conclusion. They found that the lethality associated with incubation of *top2* mutants at their restrictive temperature is cell cycle dependent and has a clear execution point, despite the fact that *top2* mutants have no uniform arrest morphology.

NDC1 Function Is Required Preferentially for Meiosis II

We draw two major conclusions from the effect of *ndc1-1* on meiosis. First, the meiosis II divisions are much more sensitive to the *ndc1-1* defect than the meiosis I division. Second, the *ndc1-1* defect appears, at least in meiosis, to be imposed either completely or not at all, frequently affecting only one of the two meiosis II spindles in the same cell. The first conclusion is tentative, since our method of assay of the meiotic products may be subject to ascertainment bias: if cells which fail in the meiosis I division also

tend to fail in meiosis II, then they would not form two-spored asci and would escape detection.

Our observations are most simply explained if ndc1-1 causes a defect in mitosis and meiosis II and not meiosis I. A major feature of chromosome mechanics that is unique to meiosis I is that the centromeres do not separate. If ndc1-1 were required to construct new functional centromeres, it might not be required for meiosis I. The spindle pole body divides in meiosis I as it does in mitosis and meiosis II, but it may not undergo duplication of its material (Byers and Goetsch, 1973). Thus the centromeres and the spindle pole body are attractive candidates for the site of action of NDC1.

The observation that the meiosis II chromosome segregation in ndc1-1 is either normal or fails completely is made more striking by the fact that the two meiosis II spindles actually lie within a single nuclear compartment only a few microns apart (Esposito and Klapholtz, 1981). Even in such close proximity, one of the meiosis II spindles can fail completely while the other acts normally. This argues against a role for the NDC1 gene product in a structure or process that could act differentially on different chromosomes (such as a microtubule), since a condition partially defective for such a structure should cause nondisjunction, resulting in aneuploidy at high levels. Indeed MBC, which depolymerizes microtubules, has such an effect in mitosis (Wood, 1982; meiosis was not tested) and a beta-tubulin mutant has that effect in mitosis and meiosis (Thomas, Ph.D. thesis, 1984; T. Huffaker, personal communication; and our unpublished observations).

Instead, this phenotype seems more compatible with a role for the NDC1 gene product as part of a process or structure that can exist in only one of two states. This might be because the NDC1 gene product has a regulatory function that determines when chromosomes in mitotic-like divisions will attach to their pole. If response to the regulation were discrete, it would result in the observed behavior. Alternatively, the NDC1 gene product may be part of a structure that is difficult to form at nonpermissive temperature but, once formed, is fully functional. Finally, the NDC1 gene product might act very cooperatively or it might be present in only one or very few copies per chromosome set.

Possible Molecular Function of the NDC1 Gene Product

We would like to suggest two speculative molecular models for the defect imposed by ndc1-1. In Model A, NDC1 is required for the production of a spindle pole body functional for attachment to chromosomes. In the ndc1-1 arrest, the previously existing ("old") spindle pole body attaches normally to the chromosomes and they segregate via this attachment. The old spindle pole body is functional because it had been acted upon by the ndc1-1 gene product in the cell cycle previous to shift to restrictive temperature. In this model, the NDC1 gene product could be a structural component of the spindle pole body, which, when defective, results in the construction of a spindle pole body defective for attachment to chromosomes. When the ndc1-1 gene product has been incorporated into the spindle pole body at permissive temperature its struc-

ture is stabilized and no longer significantly cold sensitive. Such a phenomenon has precedent in studies of phage morphogenesis, in which conditionally mutant proteins are typically conditional for assembly, but once incorporated into a structure are functionally stable (for example, Casjens and King, 1975). Alternatively, the function of NDC1 could be catalytic, causing a permanent modification of each new spindle pole body that renders it competent for spindle formation.

In Model B, NDC1 is required for the synthesis of new functional centromeres. In ndc1-1 cells at nonpermissive temperature only the previously existing centromere for each chromosome is competent to attach to the spindle, resulting in the segregation of both chromatids to one pole. Mechanisms similar to those described for Model A could account for retention of function by the "old" centromere. This model requires that the 16 or 17 chromosomes of yeast (Mortimer and Schild, 1982) segregate with a high degree of cooperativity ("old" centromere structures segregate together). There is evidence based on studies of DNA replication (Williamson and Fennell, 1981) that chromosome sets of the same replicative age cosegregate in mitosis in yeast, consistent with a cooperative mechanism of disjunction. If the NDC1 product does act at the centromeres, our results would support models of chromosome segregation in which the centromeres become attached to a common structure for segregation at mitosis.

These models can be most easily distinguished from each other by the molecular identification and analysis of the NDC1 gene and its gene product by the methods of molecular biology.

Experimental Procedures

Strains and Genetic Methods
Media, methods of mating, and tetrad analysis were as described by Sherman et al., 1974. The can1, cyh2, and cyr1 alleles used here were all derived as spontaneous mutants selected on the appropriate drug (canavanine sulfate, Sigma Chemical Co., 50 μg/ml in SD medium lacking arginine; cycloheximide, Sigma Chemical Co., 1 μg/ml in YEPD; cryptopleurine, Chemasea Manufacturing Pty. Ltd., Sidney, Australia, 0.1 μg/ml in YEPD).

Isolation of ndc1-1 from the Mutant J76
The cold-sensitive strain J76 was isolated by D. Moir as described in Moir et al. (1982). These authors do not discuss the mutant J76, because of its genetic intractability; however, they recognized the mutant as a cold-sensitive Cdc-like mutant. Moir found that when J76 was crossed to wild-type haploid strains and tetrads were analyzed, aberrant patterns of segregation of parental markers were observed. For the studies described here, we succeeded in eliminating the genetic anomalies associated with J76 by extensive backcrossing to wild-type strains. J76 (MATa his4 Cs⁻) was crossed to DBY1489 (MATa ade2 lys2 trp1 Cs⁺) and the resulting diploid was sporulated and subjected to tetrad analysis. The viability of the spores was low (less than 50%), and the patterns of marker segregation in the testable spore clones suggested that the J76 parent was diploid. About 50% of the spore clones were cold sensitive. We repeatedly backcrossed the mutation responsible for the cold sensitivity of J76 to wild-type haploid strains in S288C background. By the fifth backcross sporulation was very efficient, the tetrads showed excellent viability, all markers, including Cs lethality, segregated 2:2, and the colonies formed by all four spores were indistinguishable in size at the permissive temperature. A Cs spore from the fifth backcross was recessive for cold sensitivity and failed to complement the original J76 isolate. By crossing ndc1-1 isolates from the fifth backcross to strains carrying markers on every chromosome, and performing tetrad analysis, we showed that the back-

crossed strains were indeed haploids. Recently A. Hoyt in our laboratory has identified two additional mutant alleles of *NDC1* whose phenotypes closely resemble the *ndc1-1* phenotype described here (personal communication).

Genetic Mapping of NDC1

Cumulative tetrad data from crosses of *ndc1-1* with the two closely centromere linked markers *leu1* and *trp1* (22:32:30, PD:NPD:TT) indicated that *NDC1* is about 20 cM from its centromere. Therefore, strains carrying *ndc1-1* were crossed to strains multiply marked by auxotrophs on several chromosomes, generally near their respective centromeres (DBY1186 *MATα spo11 ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1*; DBY1187 *MATa spo11 ura3 his2 leu1 lys1 met4*; and DBY1189 *MATa spo11 ura3 ade1 his1 leu2 lys7 met3 trp5*, provided by S. Klapholtz), and tetrad analysis was performed. Statistically significant linkage was found with only one of the 19 genes tested, *lys7* on chromosome 13 (8:1:14, PD:NPD:TT, suggesting a distance of roughly 50 cM). The location of *NDC1* on chromosome 13 was confirmed by a cross to *cdc5* (DBY1596 *MATa cdc5 his7 ura1*, provided by G. Fink), which indicated that *NDC1* is about 20 cM from *CDC5* (19:0:12, PD:NPD:TT). Since *NDC1* is centromere linked, we inferred that it must lie on the right arm of chromosome 13, near the *RAD52* gene. We therefore made a cross in which *cdc5*, *rad52*, and *ndc1* were segregating (*MATa cdc5 ndc1-1* × DBY1596 *MATα his4-539 leu2-3 lys2-52 rad52-1*, provided by G. Fink). *NDC1* and *RAD52* proved tightly linked (23:0:0, PD:NPD:TT). Recently A. Hoyt (personal communication) has shown that *NDC1* and *RAD52* are physically adjacent genes.

Cell Cycle Arrest

Cells were grown to 2–5 × 10^6 cells/ml under permissive conditions in liquid YEPD and shifted to the nonpermissive temperature by placement in a rotary water bath of the appropriate temperature. Microscopic observations were made following sonication sufficient to separate cells without breaking a significant fraction of them. The "number of generation times" a mutant strain was incubated at its restrictive temperature refers to the generation time of wild-type strains at this temperature. In the case of *ndc1-1* the actual generation time of the mutant at its permissive temperature was within 10% of wild-type. The generation time of DBY1398 and other haploid yeast strains of related genetic background is 8–10 hr at 13°C, and about 12 hr at 11°C.

Arrest on Solid Medium

In order to follow the arrest of individual cells on solid medium, a modification of the method of Hartwell et al. (1970) was used. Two sterile squares of window screen (about 1 cm square) were placed on a microscope slide cleaned with ethanol, and about 2 ml of YEPD + 2% agar was pipetted onto the slide, resulting in a thin layer of nutrient agar with immovable marker squares embedded in it. Cells growing in liquid YEPD at permissive temperature were sonicated lightly to separate daughters, 4.5 μl of an appropriate dilution of cells was spotted onto the agar above each square of screen, and a coverslip was immediately placed over the liquid spot. This resulted in good optical resolution of cells without inhibiting their growth. When viewed at 160× magnification each hole in the screen roughly filled the microscope field. Photographs of several fields from each sample were taken and the slide was shifted to 11°C. At time intervals the slide was removed briefly (2–3 min) to room temperature for photography of the same fields and returned to 11°C. We demonstrated that this transient removal from 11°C did not affect the kinetics or the final result of the *ndc1-1* arrest by comparing *ndc1-1* samples that were repeatedly removed and photographed over a long time course with a set of samples, each of which was photographed only twice, once at the time of shift and again at one time point. The data presented in Results is for samples that were photographed at several time points because this provided more information.

Determination of Ploidy

Ploidy of the strains was tested in two ways, a "quick screen," to which all segregants were subjected, and a more extensive analysis of ploidy of several of the strains, which in all cases (11/11) confirmed the reliability of the quick screen. For the quick screen the strains to be tested (canavanine-sensitive strains) were crossed by replica plating to a lawn of a haploid strain that carried a recessive canavanine-resistance marker (DBY1511 *MATα ura3-52 hom3 leu2 lys2-801 can1*). After mating

and growth overnight on rich medium, cross progeny were selected by replica plating to an appropriate minimal medium, and allowed to grow overnight and replica plated to canavanine medium, while retaining selection for cross progeny. Care was taken throughout this procedure to transfer comparable numbers of cells for each strain tested. In the final replica to canavanine, several serial replicas were performed, with the effect of transferring varying numbers of cells to the plates. Control haploid and diploid strains were included on each plate. Whenever the strain being tested was haploid 20–50 mitotic recombinants that had homozygosed the Can^R marker by mitotic recombination (thereby becoming resistant to canavanine) were observed after 2 to 3 days. When the strain was diploid no single mitotic recombination could give rise to a Can^R segregant, and no Can^R papillae arose on the selective plate.

Although reliable, the quick screen actually only tested the number of copies of chromosome 5 (on which *CAN1* resides). Therefore the Can^R test was confirmed in 11 cases by crossing with haploid and diploid tester strains, and dissecting tetrads from each cross. Putative haploid isolates were crossed to a haploid tester strain and high spore viability and 2:2 segregation of markers confirmed that it was indeed a haploid. Putative diploid isolates were crossed with a tester diploid, and high spore viability and the characteristic tetraploid pattern of marker segregation confirmed that the isolate was indeed diploid. Furthermore, in each case, the four spores from at least one tetrad that segregated four nonmater spores, were resporulated and shown to be diploids. Even these tetrad tests do not rule out a very limited number of extra chromosomes in the strain to be tested, although they do rule out any shortage of chromosomes (for instance a 2N–1 genotype).

Hydroxyurea and Alpha-Factor Treatments

Hydroxyurea (Sigma Chemical Co.) arrest in liquid medium was performed as described by Hartwell (1976). For alpha-factor (synthetic alpha-factor, Sigma Chemical Co.) arrests *MATa* cells were grown to 1–2 × 10^7 cells/ml in YEPD medium that had been adjusted to pH 4.0 with HCl and filtered for sterilization. The cell concentration was adjusted to 1 × 10^7 by resuspending in the appropriate volume of YEPD (pH 4.0), and alpha-factor was added to a concentration of 2 or 4 μg/ml. To achieve good arrest 2 μg/ml was just sufficient, and for longer arrests 4 μg/ml was used to minimize eventual leakage through the block.

Fixation of Cells for DAPI Stain

A sample of 1–2 ml of cells was removed from a culture of logarithmic phase cells. The cells were centrifuged, washed once with 0.85% NaCl, resuspended in 2 ml of methanol : acetic acid (3:1, vol:vol), allowed to fix at room temperature for 30 min or longer, then centrifuged and washed twice with 10 mM Tris (pH 7.5), 0.85% NaCl. The washed cells were sonicated briefly to disrupt clumps. To these cells, DAPI (4'-6'diamidino-2-phenylindole, Accurate Chemical Co.) was added to 1 μg/ml, and incubated at room temperature for at least 5 min. The DAPI was washed out one to three times with 0.85% NaCl for viewing. The cells were viewed and photographed on a Zeiss standard microscope equipped for epifluorescence. Both phase contrast and fluorescence images were viewed with a Zeiss Neofluor 100× N.A. 1.3 objective under oil immersion. DAPI was viewed using a Zeiss selective filter set 487702–7960.

Immunofluorescence, Microscopy, and Photography

The fixation, preparation, and antibody staining were performed as described for formaldehyde fixation by Kilmartin and Adams (1984), with the following modifications: cell walls were removed by incubation at 30°C for 1 hr with gentle rocking in 1.2 M sorbitol, 0.1 M KP_i (pH 7.5), 25 mM beta-mercaptoethanol with Zymolyase 60,000 (Miles Laboratories Inc.) added to 50 μg/ml. DAPI was added to the mounting medium at 0.2 μg/ml. Cells were viewed and photographed as described for DAPI stain, but using Zeiss selective filter set 487715–9902. Photography was with Kodak Tri-X Pan film, developed with either Diafine or Microdol X.

Fixation of Cells for Calcafluor and DAPI Double Stain

Cells were fixed in formaldehyde as for immunofluoresence and were washed twice with water. The cell pellet was resuspended in 50 mM KP_i (pH 7.0), 0.1% Triton X-100, 10 μg/ml Calcafluor White (a generous gift from J. Pringle), 0.2 μg/ml DAPI, and 0.1% phenethylenediamine

(Sigma Chemical Co.). Under these conditions most cells are visibly stained for their chitin rings and for their nuclear DNA, although the fluorescent background is high and most cells were not unambiguously interpretable. DAPI and Calcafluor were observed with the same filter set.

Quantitative Sporulation

Strains to be sporulated were patched on YEPD plates and grown overnight at permissive temperature at a cell density that produced a thin layer of growth in which few of the cells were starved. A large sample of cells was removed from the patch with a wooden dowel and evenly patched onto an agar plate containing 1% potassium acetate that was preequilibrated to the experimental temperature (and/or contained the appropriate drug). Thus the shift to conditions that induced sporulation was simultaneous with temperature shift or the shift to drug conditions. This procedure, in control strains, consistently produced sporulation levels of 40%–60%, nearly as high an efficiency of sporulation as that achieved by optimal liquid sporulation procedures (50%–70% sporulation, Davidow et al., 1980), and was more convenient for the many temperatures of sporulation employed.

Dissection of Two- and Three-spored Asci

A method similar to that of Davidow et al. (1980) was used to dissect asci containing fewer than the normal four spores. Sporulated cells were resuspended in water and applied in a line bisecting a YEPD plate using a sterile 25 μl capillary pipette, and a 5 mg/ml solution of Zymolyase 60,000 in 1.0 M sorbitol, 50 mM KP$_i$ (pH 7.5) was applied by the same method in an adjacent line. Two- or three-spored asci were tentatively identified by observation in a dissecting microscope and micromanipulated to a predetermined position overlying the Zymolyase soaked region of the plate. The asci were digested for 10–20 min and their identity confirmed by counting the number of spores released from the ascus wall. The spores were micromanipulated to predetermined positions on the agar and allowed to grow into spore clones (spore viability for wild-type strains was >95%). Remaining analysis was the same as for other tetrad analysis. We were unable to dissect two-spored asci from wild-type sporulated at low temperatures because too few were produced. However, comparison of the number of two-spored asci produced by wild-type and ndc1-1 strains at 11°C indicates that the background of two-spored asci not caused by the ndc1 defect must be low.

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