NDC1: A Nuclear Periphery Component Required for Yeast Spindle Pole Body Duplication

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Abstract. The spindle pole body (SPB) of Saccharomyces cerevisiae serves as the centrosome in this organism, undergoing duplication early in the cell cycle to generate the two poles of the mitotic spindle. The conditional lethal mutation ndcl-1 has previously been shown to cause asymmetric segregation, wherein all the chromosomes go to one pole of the mitotic spindle (Thomas, J. H., and D. Botstein. 1986. Cell. 44:65-76). Examination by electron microscopy of mutant cells subjected to the nonpermissive temperature reveals a defect in SPB duplication. Although duplication is seen to occur, the nascent SPB fails to undergo insertion into the nuclear envelope. The parental SPB remains functional, organizing a monopolar spindle to which all the chromosomes are presumably attached. Order-of-function experiments reveal that the NDC1 function is required in (31 after a-factor arrest but before the arrest caused by cdc34. Molecular analysis shows that the NDC1 gene is essential and that it encodes a 656 amino acid protein (74 kD) with six or seven putative transmembrane domains. This evidence for membrane association is further supported by immunofluorescent localization of the NDC1 product to the vicinity of the nuclear envelope. These findings suggest that the NDC1 protein acts within the nuclear envelope to mediate insertion of the nascent SPB.

Assembly of the mitotic spindle in a eucaryotic cell is dependent on the formation of two centrosomelike organelles from which spindle microtubules emanate. The apparent duplication of centrosomal components to generate spindle poles is well described cytologically (reviewed by McIntosh, 1983; Brinkley, 1985; Sluder, 1989), but few molecular details of the underlying mechanism are known. The relevant organelle in the yeast Saccharomyces cerevisiae is the spindle pole body (SPB), which is situated within the nuclear envelope (reviewed by Winey and Byers, 1992). As the sole microtubule organizing center in S. cerevisiae, the SPB forms microtubular arrays in both the cytoplasm and the nucleus. Electron microscopy of wild-type and mutant yeast strains has permitted description of the SPB duplication pathway and spindle formation (Byers, 1981a; Winey et al., 1991). A crucial early step in this pathway is the formation of the satellite on the outer surface of the half-bridge structure adjacent to the extant SPB. At START in G1, the satellite-bearing SPB is transformed into duplicated, side-by-side SPBs, both of which are inserted into the nuclear envelope and bear both nuclear and cytoplasmic microtubules. This G1 duplication event is thought to occur by a conservative mechanism where the satellite structure serves as the precursor for the new SPB, and the existing SPB from the previous cell cycle remains intact. Recently, Vallen et al. (1992) clearly demonstrated that a KARI-BGal fusion protein is localized to only one of the newly duplicated SPBs, providing direct evidence that SPB duplication is indeed a conservative process. Separation of the two SPBs occurs later, leading to assembly of the bipolar mitotic spindle.

The various stages of SPB duplication described above for wild-type cells are also observed in cdc- (cell division cycle) mutants at their terminal arrest points. Satellite-bearing SPBs are observed in yeast cells arrested in G1 by mating pheromone or mutations in the CDC28 gene, whereas duplicated side-by-side SPBs are observed in cells arrested later in G1 by cdc4 and cdc34 (Byers and Goetsch, 1974, 1975; Goebl et al., 1988). Other mutants are specifically defective in SPB duplication. Cells bearing mutations in the CDC31 or KARI (karyogamy) genes fail to undergo SPB duplication altogether despite execution of other cell cycle functions. This leads to arrest as a large budded cell with G2 DNA con-
Materials and Methods

**Strains, Cell Culture, and Genetic Techniques**

The yeast strains used in this study are listed in Table I. Yeast media and genetic techniques were as described by Hartwell (1967) and Sherman et al. (1971). The ndc-4 allele was isolated as an ndc-1 noncomplementing mutation. The ndc-1 allele is cold-sensitive (cs-) for growth at 14°C. The mutant screen for ndc-1 noncomplementing mutations was carried out essentially as described by Stearns and Botstein (1988). A stationary phase culture of DBY1399 (Table I) was mutagenized using ethylmethane sulfonate (EMS). Mutagenized colonies were recovered on YEPD plates after 3 d at 26°C and were mated at 26°C with cells of DBY1583 and DBY1584 (Table I). Diploid products of both crosses were selected at 14°C on minimal medium (SD) supplemented with uracil. Putative noncomplementors of ndc-1 were identified as single transductants from a genomic library in a URA3-CEN vector (Rose et al., 1987). Cells were shifted to the nonpermissive temperature (36°C) for growth phenotype. Tetrad analysis showed that the ts- phenotype is tightly linked to NDC1, suggesting that this phenotype is caused by a mutation in NDC1. Furthermore, the ts- phenotype of ndc-4 can be complemented by a CEN plasmid that contains the wild-type NDC1 gene. Thus, we conclude that the noncomplementation and the ts- phenotypes are both caused by the ndc-4 mutation.

Yeast cells were arrested in G1 with α-factor (7–10 μM) produced by custom peptide synthesis using F-MOC chemistry on a peptide synthesizer (model 488, Applied Biosystems Inc., Foster City, CA). The efficiency of a given arrest was monitored by determining the budding index (proportion of budded cells in a sample of 200 cells) of briefly sonicated aliquots. Arrested cultures were considered adequate when 95% of the cells were unbudded, and the arrest was later confirmed by flow cytometry (see below) to show that the population was predominantly comprised of cells with G1 DNA content. Cells arrested by treatment with α-factor or by the cdc34-2 mutation at the nonpermissive temperature (36°C) were released from these blocks by rinsing twice in growth medium equilibrated to the appropriate temperature for the experiment. In these experiments, entry into and progression through the cell cycle were monitored by budding index and flow cytometry.

**Isolation and Characterization of the NDC1 Gene**

Yeast strain DBY1583 (ndc-1, Table II) was transformed with plasmid DNA from a genomic library in a URA3-CEN vector (Rose et al., 1987). Cells transformed to uracil prototrophy were obtained at 30°C and replica transferred to minimal media (SD) minus uracil plates at 11° and 14°C. The plasmid DNA from three cold-resistant transformants was transferred to E. coli for further analysis. All three were found to contain overlapping regions of chromosomal DNA (see Results). The region encoding the NDC1 complementing activity was identified by subcloning smaller fragments into yeast vectors and reintroduction into DBY1583. In addition, the bacterial transposon γδ was used to create disruptions of a plasmid-carried NDC1 gene using the protocol of Guyer (1978). The DNA comprising nucleotides −300 to +2258 (Fig. 3) was sequenced on both strands using sequential overlapping clones produced by the method of Dale et al. (1985) The resulting M13 clones were sequenced with a kit designed for this purpose (American Corp., Arlington Heights, IL) following the instructions provided by the supplier. Analysis of the DNA sequence of the NDC1 gene and its derived amino acid sequence was carried out using programs in the Eucgene.
Strains after Release from Various G1 Arrests

| G1 arrest (SPB state) | Release temperature | Spindle† | SPB‡ |
|-----------------------|---------------------|---------|------|
| α-factor (satellite-bearing SPB) | 25°C | 162 | 0 |
| cdc34** (duplicated side-by-side SPBs) | 15°C | 118 | 5 |

† SPB morphology in arrested cells was confirmed by electron microscopy.‡ Spindle morphology determined by immunofluorescent staining of microtubules.

Table II. Spindle and SPB Morphologies in ndc1-1

Cytological Techniques

Cytological experiments were carried out using diploid strains because their larger SPBs and spindles are easier to visualize. Immunofluorescent staining of microtubules was carried out as described by Kilmartin and Adams (1984) as modified by Jacobs et al. (1988) using the rat mAb YOL1/34 (anti-α-tubulin) and FITC-conjugated goat anti-rat antibodies (Accurate Chemical and Scientific Corp., Westbury, NY). DNA was stained with DAPI (1.0 μg/ml; Sigma Chemical Co., St. Louis, MO) for the subcellular localization of Ndc1p, formaldehyde-fixed yeast spheroplasts (DBY1826/1829 carrying pRB1239, Table I) were stained first with affinity-purified rabbit anti-Ndc1p antibodies (Pringle et al., 1989), then with affinity-purified goat anti-rabbit IgG (Zymed Laboratories, Inc., South San Francisco, CA), and finally with FITC-conjugated affinity-purified rabbit anti-goat IgG (Organon Teknika Corp., West Chester, PA). Stained cells were viewed with a Nikon Microphot FX fluorescence microscope and photographed with Kodak Kodachrome 200 Professional film, or viewed with a Zeiss Axioskop fluorescence microscope and photographed with Kodak Type 2415 Technical Pan high sensitivity film (Lumicon, Livermore, CA).

Results

The NDC1 Gene Is Required for Spindle Pole Body Duplication in G1

Strains mutant for NDC1, when shifted to the nonpermissive temperature (15°C for ndc1-1, Thomas and Botstein, 1986; or 37°C for ndc1-4, see Materials and Methods), segregate all their chromosomal DNA to one spindle pole, yielding one cell with twice the original ploidy and another cell that is haploid. Similar phenotypes have also been observed as a result of the monopolar mitosis of other mutants that are defective in SPB duplication (reviewed by Winey and Byers, 1992). Fluorescence microscopy of ndc1-1 cells subjected to the nonpermissive temperature and stained with antibodies

Figure 1. Immunofluorescent staining of ndc1-1 containing strain DBY1503-1 (Table I, see Materials and Methods). A late mitotic cell at the permissive temperature (25°C) with a long spindle (FITC) and separated DNA (DAPI) is shown. At the nonpermissive temperature (15°C), two distinct foci of microtubules which do not appear to be connected by a mitotic spindle (FITC) are observed, and the DNA is not separated (DAPI).

Antibody Production

Plasmid pCC119 was constructed by inserting a 2.5-kb Sppl/HindIII DNA fragment that contains most of the NDC1 gene into the Smal/HindIII sites of the TrpE fusion protein vector pATH10 (Broach et al., 1991). This construction created an in-frame fusion between TrpE and NDC1. The TrpE-NDC1 fusion protein was partially purified from E. coli cells harboring pCC119 (Broach et al., 1991) and was further purified on a preparative SDS-polyacrylamide gel for use as antigen in injections of rabbits. Immune sera were adsorbed three times against heat-treated total E. coli extract prepared from cells carrying pATH10. The E. coli-depleted sera were then used for affinity-purification of anti-Ndc1p antibodies by adsorption against partially purified (as above) TrpE-NDC1 fusion protein that was immobilized on a nitrocellulose membrane (Pringle et al., 1989).

For immunoblotting experiments, whole control plasmid pRB307 or the high-copy number NDC1-containing plasmid pRB1239. Proteins were transferred from an SDS-polyacrylamide gel to a nitrocellulose filter (Schleicher and Schuell, Keene, NH) as described by Burnette (1983). To detect the NDC1 gene product (Ndc1p) on the nitrocellulose filter, affinity-purified anti-Ndc1p antibodies were used as primary antibodies that were subsequently detected by 125I-protein A (New England Nuclear, Cambridge, MA).

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Figure 2. Electron microscopic analysis of ndc1-1 (DBY1503-1) and ndc4-4 (Dndc-4) containing strains (Table I, see Materials and Methods). B shows a normal mitotic spindle observed in DBY1503-1 at the permissive temperature. The arrows indicate SPBs, large carets highlight spindle microtubules, and nuclear pores are indicated by small carets in other panels. Defective SPBs (arrows) adjacent to the functional SPB found in DBY1503-1 at the nonpermissive temperature (14°C) are shown in A and C. A representative separated monopolar spindle (E) and defective SPB (D) found in DBY1503-1 at the nonpermissive temperature (14°C) is shown. Identical morphologies of a monopolar spindle (G) and defective SPB (F) are observed in a ndc4-4 mutant cell at its nonpermissive temperature of 36°C. These morphologies have been observed in no less than 15 serially sectioned nuclei of each mutant. Bars, 0.1 μm; and A, C–F are at the same magnification as B.
specific for tubulin shows that the large budded cells contain two microtubule organizing centers, but the associated microtubules do not appear to form a normal mitotic spindle (Fig. 1). Electron microscopic observation clarifies the nature of the defect in ndcl mutants at nonpermissive conditions. Examination of serial thin-sections reveals that each large budded cell contains one SPB of normal appearance and a second one that is defective (Fig. 2). The normal SPB bears microtubules on both its nuclear and cytoplasmic faces, whereas the defective SPB has microtubules only on the cytoplasmic face. In most cells, the defective SPB undergoes separation from the functional SPB and is often located at the end of a thin projection of the nuclear envelope (Fig. 2 F).

The structural defect observed in ndcl mutants suggests a specific role for the gene during SPB duplication. To assess the nature of this requirement, we synchronized ndcl-1 cells at two different points in the G1 phase of the cell cycle before release at either permissive or nonpermissive temperatures. One type of G1 arrest was achieved by treatment with a-factor, which blocks progression at the stage with a satellite-bearing SPB (Byers and Goetsch, 1975). The other G1 arrest used was caused by the cdc34-2 mutation, wherein mutants transferred to the nonpermissive temperature are blocked at a stage with duplicated side-by-side SPBs (Goebel et al., 1988). An a-factor sensitive diploid strain homozygous for ndcl-1 (a/a, DBY1503-1, Table I) was arrested with a-factor at 25°C, then released from this arrest at 14°C to inactivate the ndcl-1 gene product or at 25°C (permissive condition). As expected, electron microscopy confirmed that these a-factor arrested cells contain satellite-bearing SPBs. Cells released from this arrest were monitored for entry into the cell cycle using light microscopy to assay bud formation and flow cytometry to observe entry into and completion of DNA synthesis. Cells released at 25°C budded and completed DNA synthesis in about 90 min. Examination by indirect immunofluorescent staining of microtubules showed that these cells contained normal mitotic spindles (as in Fig. 1, see Table II). Cells released at 14°C, on the other hand, exhibited the ndcl phenotype described earlier (Table II). These observations were confirmed by electron microscopic analysis of the same cultures. Cells released at 25°C were found to contain normal spindles and SPBs (as in Fig. 2 B; Table II), whereas those released at 14°C contained the characteristic monopolar spindle and the defective SPB (as in Fig. 2 A, C-G; Table II). Among the cells released at 14°C and viewed by electron microscopy, only one cell of 48 examined was found to contain a normal mitotic spindle. The vast majority of cells (47/48) had uniformly suffered the defect in SPB duplication. We conclude that the ndcl gene function is required at a stage of SPB duplication that follows the satellite-bearing SPB stage.

In a second experiment, the requirement for ndcl function after completion of SPB duplication was tested. A strain (Dndc/34, Table I) doubly mutant for cdc34 and ndcl was brought to the cdc34 arrest by incubation at 36°C. Cells arrested in this manner contain duplicated side-by-side SPBs, a phenotype that was confirmed by electron microscopy. The cells were then released from this arrest by transfer either to a temperature permissive for both mutations (25°C) or to the nonpermissive temperature for only the ndcl-1 mutation (14°C). Cell cycle progression upon release from the cdc34 block was again monitored by flow cytometry, and spindle structure was monitored both by immunofluorescent staining of microtubules and by electron microscopy. Release of these cells from the cdc34 block at either temperature led to the formation of normal mitotic spindles (Table II), demonstrating that the ndcl function is not required either for separation of the SPBs or the subsequent formation of the mitotic spindle.

**NDC1 Encodes an Essential, Hydrophobic Protein**

The ndcl gene was isolated by complementation of the ndcl-1 cold-sensitive phenotype. Plasmids that conferred on ndcl-1 strain DBY1583 the ability to grow at 11°C were isolated from a centromere vector-based genomic library (Rose et al., 1987). Restriction enzyme sites were mapped in the genomic DNA inserts from three such plasmids, and restriction fragments common to all three plasmids were identified. Tight genetic linkage between NDC1 and the DNA damage repair gene RAD52 had been reported (Thomas and Bot-
stein, 1986), so the observation of common restriction fragments between the ndcl-suppressing plasmids and YEp13-X70281 (Id. SCNDCIA) was not unexpected. Therefore, the DNA insert in these plasmids corresponds to the RAD52 and NDCl loci on chromosome XIII (Mortimer et al., 1989). The ndcl-complementing activity was further localized within the isolated genomic DNA by subcloning and by bacterial transposon \( \gamma \delta \) mutagenesis (Fig. 3). These experiments and DNA sequencing of this region revealed that the reading frames of the NDCl gene and the adjacent RAD52 gene lie in opposite orientation with proximal 5' ends (Fig. 3). The 5' end of the then unidentified NDCl gene had been detected and partially characterized by Adzuma et al. (1984) in their analysis of the RAD52 locus. As shown in that study, the transcription of NDCl initiates ~45 bp upstream of the predicted NDCl start codon.

The NDCl gene was disrupted to determine the effect of a null allele on viability. Haploid strains were constructed (i.e., MA1998, Table I) that carried wild-type alleles of NDCl and URA3 on a plasmid and the ndcl:LEU2 disruption and ura3-52 alleles at their respective chromosomal loci. At temperatures ranging from 11-37°C, these strains were unable to segregate cells resistant to 5-fluoro-orotic acid, a compound that inhibits growth of URA3\(^+\) cells (Boeke et al., 1984). This result indicated that these strains required the continued presence of the NDCl-containing plasmid for viability and therefore that the NDCl gene is essential for mitotic viability. The derived 656 amino acid sequence (predicted molecular weight of 74 kD, see Fig. 4) of the NDCl gene was compared to all amino acid sequences in GenBank (Release 74.0), and no protein with significant similarity was detected. A hydropathy plot for the NDCl amino acid sequence is shown in Fig. 5, and stretches of amino acids that appear hydrophobic according to the algorithm of Kyte and Doolittle (1982) are indicated. These regions were also examined by the algorithm of Eisenberg et al. (1984) as shown in Table III. Six or seven regions of the predicted NDCl protein are of sufficient length and hydrophobicity to be membrane spanning segments. The mean hydrophobic moment of each putative transmembrane segment (Table III) suggests that these regions could interact with each other, or interact with the transmembrane domains of other integral membrane protein(s) (Eisenberg et al., 1984).

Figure 5. Kyte-Doolittle hydrophathy plot of the NDCl encoded protein, where the average hydrophobicity of each amino acid was calculated using a window of nine amino acids. The regions with positive values are more hydrophobic. The positions of the seven possible transmembrane domains are shown as bars above the plot. Also shown is the position of the alternate (A) transmembrane domain, which spans putative transmembrane domains 3 and 4. Detailed information on these domains is presented in Table III, which uses the same nomenclature.

Table III. Characteristics of Predicted Transmembrane Domains of the NDCl Protein

| Transmembrane Segment* | Kyte-Doolittle\( <\phi> \) | Eisenberg\( <\H> \) | \( <\mu_d> \) |
|------------------------|-----------------|-----------------|---------|
| 1                      | 34–52           | 2.3             | 34–54   | 0.65 | 0.23 |
| 2                      | 61–79           | 2.4             | 59–79   | 0.79 | 0.23 |
| 3                      | 92–110          | 1.5             | 90–110  | 0.55 | 0.17 |
| 4                      | 117–135         | 1.4             | 117–137 | 0.55 | 0.30 |
| 5                      | 192–210         | 2.4             | 191–211 | 0.73 | 0.22 |
| 6                      | 225–243         | 2.3             | 225–245 | 0.71 | 0.18 |
| 7                      | 514–532         | 1.6             | 513–533 | 0.51 | 0.23 |
| Alternatel             | 112–130         | 1.7             | 110–130 | 0.67 | 0.26 |

* These predicted transmembrane segments are underscored in Fig. 4, and are shown in Fig. 5.
\( \dagger \) The average hydropathy (\( <\phi> \)) of 19 amino acid segments was calculated using the hydropathy index of Kyte and Doolittle (1982).
\( \ddagger \) The mean hydrophobicity (\( <\H> \)) was calculated across 21 amino acids using the hydrophobicity index of Eisenberg et al. (1984). The mean hydrophobic moment (\( <\mu_d> \)) for these 21 amino acid segments was calculated by using the \( \mu_d \) for each amino acid in the algorithm of Eisenberg et al. (1984). An alternate transmembrane segment can be identified that would replace transmembrane segments 3 and 4, yielding a model with six transmembrane segments.

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Figure 6. Specificity of affinity-purified anti-Ndclp antibodies. Roughly equal amounts of yeast whole cell lysates prepared from DBY1826 carrying the control plasmid pRB307 (lane 2) or the high copy number NDC1 plasmid pRB1239 (lane 1) were used for immunoblotting with affinity-purified anti-Ndclp antibodies. The arrowhead shows the detected Ndclp. With longer exposure of the autoradiogram, protein species of much higher apparent molecular weights are also detected in cells containing pRB1239.

The NDC1 Gene Product Localizes to the Vicinity of the Nuclear Envelope

To better understand the cause of the cytological defect seen in ndcl mutants, we determined the localization of the NDC1 gene product (Ndclp). Affinity-purified anti-Ndclp antibody used in an immunoblotting experiment recognized a single protein from a wild-type yeast extract (Fig. 6, lane 2). This protein, which migrates on SDS-polyacrylamide gels with an apparent molecular weight of about 62 kD, is present in greater abundance in yeast cells that carry the NDC1 gene on a high copy number plasmid (Fig. 6, lane 1).

Monospecific affinity-purified anti-Ndclp antibodies were used to determine the subcellular localization of Ndclp by indirect immunofluorescence of yeast cells. In initial tests using FITC-conjugated goat anti-rabbit IgG as secondary antibody, very weak Ndclp staining that appeared to be perinuclear was detected in some cells. This staining pattern was more readily observed in cells carrying the NDC1 gene on a high copy-number plasmid. As an added improvement, an extra antibody amplification step was added to the immunofluorescence procedure (see Materials and Methods). This latter modification yielded more consistent Ndclp staining, especially in cells carrying the NDC1 gene on a high copy number plasmid.

The characteristic pattern of Ndclp staining seen in cells carrying the NDC1 2-μm plasmid is shown in Fig. 7. Here, as in other cells, the immunofluorescent staining generally overlaps the boundary of a phase dark area, which is known from DAPI staining to be occupied by the nucleus. The intensity of this staining varies between cells, perhaps because of variation in copy number of the NDC1 plasmid. The perinuclear nature of Ndclp staining was especially obvious when different focal planes of stained cells were examined. In a small proportion of cells, Ndclp staining could be seen to extend outward from the nuclear periphery (Fig. 7 E), perhaps representing either the ER or simply a protruding portion of the nucleus. The distribution of Ndclp staining is therefore quite similar to the staining pattern seen for yeast nuclear pore components (Davis and Fink, 1990; Nehrbass et al., 1990; Wente et al., 1992), consistent with the possibility that Ndclp is a constituent of the nuclear envelope, although localization to the ER is also possible.

Discussion

We have shown here that the NDC1 gene encodes an essential, 656 amino acid protein with a calculated molecular weight of 74 kD. Found within the sequence are several stretches of hydrophobic amino acids that could be trans-

Figure 7. Localization of Ndclp in yeast cells carrying the high copy-number NDC1-plasmid pRB1239. Fluorescence images of cells stained with DAPI (A and D) and affinity-purified anti-Ndclp antibodies (B and E) and phase contrast images (C and F) of the same cells are shown.
membrane domains as defined by the methods of both Kyte and Doolittle (1982) and Eisenberg et al. (1984). The suggested association of the protein with a membrane is further supported by the localization of the \textit{NDC1} gene product by immunofluorescence microscopy. Ndclp is found in greatest concentration in the immediate vicinity of the nuclear envelope, where it presumably performs its role in SPB duplication. This localization should be considered tentative because Ndclp could only be reliably detected when over-expressed from a 2\,-\mu m plasmid. The localization of Ndclp to the entire nuclear envelope and perhaps the ER is thought to be accurate, but may be a result of over-expression.

Other than displaying the overall pattern of hydrophobicity typical of integral membrane proteins, the \textit{NDC1} gene product appears not to share striking homology with any known class of membrane proteins. Several yeast proteins, encoded by the \textit{NPSI}, \textit{NUPI}, \textit{NUPI49}, \textit{NUPI00}, and \textit{NUPI16} genes (Hurt, 1988; Nehrbass et al., 1990; Davis and Fink, 1990; Wente et al., 1992), have been previously localized to the nuclear envelope, and are thought to be peripheral membrane components of the nuclear pore complex. The putative \textit{NDC1} protein bears no resemblance to the amino acid sequences of any of these gene products, nor to any component of the nuclear pore complex of mammalian cells for which sequence data is available (reviewed by Miller et al., 1991), or to members of the large family of receptor molecules that also possess seven transmembrane domains (reviewed by Dohlman et al., 1991).

The specific type of failure in SPB duplication leading to formation of a monopolar spindle and a defective SPB, as seen here for \textit{ndcl} mutants, had previously been observed as part of the characterization of an \textit{mps2} mutant (Winie et al., 1991). That analysis suggested that \textit{mps2-1} identified a new step in SPB duplication on the basis of its novel phenotype and the results of order-of-function experiments. It was evident from that work that the \textit{MPS2}-dependent step in SPB duplication occurs at a late stage of the process, but probably before duplication is complete. However, no methods were available to test this viewpoint adequately. It is now apparent from the very similar phenotypes observed for \textit{mps2} and \textit{ndcl} mutants at their respective nonpermissive temperatures that both genes may be required for the same step in SPB duplication. We have been able to show that \textit{NDC1}, like \textit{MPS2}, is required after release from \alpha-factor arrest. The timing of the putative \textit{MPS2}/\textit{NDC1} dependent step relative to the point of \textit{cdc34} arrest was uniquely accessible in the case of \textit{ndcl-1} because it is a cold-sensitive mutation. Successful completion of the SPB duplication cycle when doubly mutant cells arrested at the \textit{cdc34} step were transferred to the nonpermissive temperature for \textit{ndcl-1} demonstrated that \textit{NDC1} is not required after SPB duplication has occurred for the SPBs to undergo separation and participate in formation of the mitotic spindle. The present experiments clearly show that \textit{NDC1} is required for the G1 transition from satellite-bearing SPB to side-by-side duplicated SPBs and render it likely that \textit{MPS2} is required in the same process.

We report here that execution of the \textit{NDC1} gene function occurs in G1, while Thomas and Botstein (1986) reported an execution point in G2. This discrepancy is resolved by examining the definition of \textit{NDC1} gene function execution in these two studies. In this study, the failed execution of \textit{NDC1} function in \textit{ndcl-1} strains has been defined by direct analysis of SPB duplication using immunofluorescence and electron microscopy. In contrast, Thomas and Botstein (1986) used diploidization (endomitosis) of \textit{ndcl-1} haploid strains as the signal that \textit{NDC1} had failed to function and inferred the \textit{NDC1} execution point on this basis. We now understand that this increase-in-ploidy assay may have revealed the execution point for endomitosis, but did not effectively report the execution point of \textit{NDC1} for SPB duplication. The endomitotic event should require not only the formation of a monopolar spindle, but also the completion of DNA synthesis, so that two sets of chromosomes would be present. The proposed dependence of endomitosis on DNA synthesis would limit the execution point for diploid formation to a point subsequent to S phase, but would not similarly constrain the execution point for SPB duplication. Our results are consistent with the model that \textit{NDC1} function is executed in G1, but endomitosis resulting from failure of \textit{NDC1} function does not occur until after DNA synthesis is complete.

The phenotype observed in \textit{ndcl} and \textit{mps2} mutants suggests that SPB duplication is a conservative process, the preexisting SPB remaining unaltered while the other is a nascent structure. In the defective processes caused by these mutations, the existing SPB evidently serves as the sole functional spindle pole while the nascent SPB is defective and plays no role in the monopolar spindle. This idea is further supported by the analysis of a \textit{KARI}-\betaGal fusion protein, which is localized to the SPB of an \alpha-factor arrested cell, but is associated with only one SPB later in the cell cycle when two SPBs are present (Vallen et al., 1992). When expression of the \textit{KARI}-\betaGal fusion gene construct in a \textit{ndcl-1} mutant at the nonpermissive temperature was examined, the fusion protein was found to be localized to what is now known to be the defective SPB. Vallen et al. (1992) suggest that the \textit{KARI} gene product is localized to the nascent SPB, and the defective SPB in \textit{ndcl-1} strains is the remnant of the nascent SPB. This interpretation is consistent with the results we have presented here.

We have demonstrated that the yeast \textit{NDC1} gene is essential and is required for the duplication of SPBs during the G1 phase of the cell cycle. Furthermore, the \textit{NDC1} gene is shown to encode a hydrophobic protein that is localized to the vicinity of the nuclear envelope. We propose that the \textit{NDC1} protein is an integral membrane protein located within the nuclear envelope where its functions include the insertion of the nascent SPB into the envelope. The \textit{NDC1} protein might play a structural role in the nuclear envelope, such as providing a site of insertion for the SPB. Alternatively, the \textit{NDC1} gene product might be involved in signaling across the nuclear envelope to coordinate activities, such as SPB duplication, that may involve functions of both the cytoplasm and the nucleus. Further analysis of the \textit{NDC1} and \textit{MPS2} genes should yield insight to the mechanism of this step in SPB duplication.

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