The Dynamics of Chromosome Movement in The Budding Yeast Saccharomyces cerevisiae

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Abstract. Nuclear DNA movement in the yeast, Saccharomyces cerevisiae, was analyzed in live cells using digital imaging microscopy and corroborated by the analysis of nuclear DNA position in fixed cells. During anaphase, the replicated nuclear genomes initially separated at a rate of 1 μm/min. As the genomes separated, the rate of movement became discontinuous. In addition, the axis defined by the segregating genomes rotated relative to the cell surface. The similarity between these results and those previously obtained in higher eukaryotes suggest that the mechanism of anaphase movement may be highly conserved. Before chromosome separation, novel nuclear DNA movements were observed in cdc13, cdc16, and cdc23 cells but not in wild-type or cdc20 cells. These novel nuclear DNA movements correlated with variability in spindle position and length in cdc16 cells. Models for the mechanism of these movements and their induction by certain cdc mutants are discussed.

Chromosome movement and spindle morphogenesis have been characterized in the budding yeast, Saccharomyces cerevisiae, by the cytological analysis of fixed cells (7, 24). In the mother cell, a short spindle forms between the spindle pole bodies (SPBs) embedded in the nuclear envelope. After DNA replication, the nucleus migrates from its location in the mother cell to the isthmus, or "neck," between the mother cell and the bud. The spindle and nuclear envelope elongate through the neck into the bud, and one genome moves into the bud while the other remains in the mother cell. Once the genomes move to the distal regions of the mother cell and bud, the spindle breaks down, the nucleus divides, and cytokinesis is completed.

A virtue to studying chromosome movement in S. cerevisiae is the ability to isolate mutants that perturb mitosis. The molecular genetic analysis of these mutants may greatly expedite the identification and characterization of the cellular components necessary for chromosome movement. In this regard, temperature-sensitive mutations in cdc13, cdc16, cdc20, and cdc23 genes are particularly interesting. At the nonpermissive temperature, these mutants arrest at a stage in the cell cycle after DNA replication: the nucleus has migrated to the neck between the mother cell and a large bud, and a short spindle has formed (24). The inability to discriminate individual mitotic chromosomes in yeast precludes directly assessing whether sister chromatids are still juxtaposed in these mutants (14). However, since the nuclear DNA appears as a single mass and the spindle fails to elongate extensively, these mutants are apparently defective in some step before or early in anaphase. Currently, it is unclear whether these mutants inhibit anaphase directly by affecting cellular components necessary for chromosome movement or indirectly by affecting other cellular processes that must be completed before yeast cells enter into anaphase.

A disadvantage to studying chromosome movement in yeast is the lack of a sensitive assay for studying chromosome movement in wild-type cells and potentially interesting mutants such as cdc13, cdc16, cdc20, and cdc23. Though the analysis of fixed cells has led to the identification of the major landmark events of mitosis, this approach has insufficient resolution for studying changes in chromosome position that occur within short intervals. Clearly, a more sensitive assay for chromosome movements involves following changes in chromosome position in live cells. In higher eukaryotes, chromosome movement can be followed in live cells with video microscopy because their chromosomes are visible with phase microscopy. Unfortunately, the inability to visualize yeast chromosomes with phase microscopy precludes this approach.

In this paper, we used digital imaging microscopy (DIM) (19) to follow the movement of fluorescently labeled nuclear DNA in live yeast cells. This new approach has allowed us to elucidate features of chromosome movement during anaphase of exponentially growing cells that were similar to those of higher eukaryotes. In addition, it revealed novel chromosome movement before anaphase in cdc16 mutants. The distribution of nuclear DNA relative to the mother cell and bud in fixed cdc16 cells not only confirmed the live cell

1. Abbreviations used in this paper: DAPI, 2,6-diamidino-phenylindole; DIM, digital imaging microscopy; SPB, spindle pole body.
observations but provided us with a simple assay for determining that this novel chromosome movement also occurred in cdc13, cdc20, and cdc23 mutants. The mechanism for generating the novel movement in cdc16 cells was addressed by additional cytological studies.

Materials and Methods

Reagents

Rabbit anti-alpha and anti-beta tubulin were gifts from Dr. Frank Solomon (Massachusetts Institute of Technology, Cambridge, MA). FITC-conjugated goat antiserum to rabbit IgG was obtained from Cappel Laboratories (West Chester, PA). Calcofluor white M2R (Cellufluor) was obtained from Polysciences, Inc. (Warrington, PA), and 2,3-diaminodiphenylindole (DAPI) was obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). Nocodazole (methyl-5-[2-thienylcarbonyl]-1-H-benzimidazole-2-yl-carbamate) was obtained from Aldrich Chemical Co. (Milwaukee, WI), and Zymolyase (100T) was purchased from ICN Immunobiologicals (Lisle, IL).

Strains

All S. cerevisiae strains were diploids and were isogenic with the strain A364A (15). The genotype of the wild-type strain was MATA/MATa leu2/LEU2 his7/His7 hom3/HOM3 ADE2/ade2 ADE3/ade3 can1/can1 sap3/sap3. For additional cytological studies, we were able to obtain time-lapse images of DNA movement in live cells. To prepare cells for microscopy, 25 ml of complete medium were inoculated with 1:1,000 vol of the appropriate saturated culture and grown overnight (23°C) sample). The remaining cells were returned to the permissive temperature, allowed to recover for 30 min, and then fixed (23/36°C sample). Since wild-type cells continue to divide at 36°C, they were shifted to 36°C at a lower cell density (10^6/ml) to ensure that the cells were fixed at the same density as the cdc mutants. Cells were fixed for fluorescence microscopy, indirect immunofluorescence, and electron microscopy using a protocol that removed the cell walls (spheroplasting) before fixation. For each strain, 5 ml of cells were spun at 2,000 rpm for 2 min, resuspended in 1 ml of SCEM medium (1 M sorbitol, 10 mM sodium citrate, 60 mM EDTA, 0.1% 2-mercaptoethanol, pH 7), and spheroplasting for 5 min at 23°C with zymolysate (final concentration 30 g/ml). Cells were fixed for 10 min by adding formaldehyde to a final concentration of 3.7% (wt/vol), spun at 5,000 rpm for 30 s, and rinsed with 1 ml of SK medium (1 M sorbitol and 50 mM KH2PO4). Cells were spun again, the supernatant was removed, and the pellet was resuspended in 70 ml of SK medium. Since cells that were arrested at 36°C were kept at 23°C for 7 min during spheroplasting, we also fixed cells at 36°C before spheroplasting. The nuclear morphology was similar for both treatments (data not shown).

Results

Nuclear DNA Movement in Exponentially Growing Cells

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**Nuclear DNA Movement in cdcl6-1 Cells**

When exponentially growing cdcl6-1 cells are shifted from their permissive temperature (23°C) to the restrictive temperature (36°C) for 3 h, >90% of the cells arrest with large buds. When these cells are then shifted back to the permissive temperature, >95% of the cells recover and reenter the cell cycle. However, in recovering cells, the frequency of chromosome nondisjunction increases (Palmer, R. E., and D. Koshland, manuscript in preparation). This result prompted us to use DIM to examine nuclear DNA movements in 18 cdcl6-1 cells that were recovering from arrest (see Materials and Methods).

While 15 of the 18 cells completed chromosome segregation, only 10 cells segregated their genomes in a pattern similar to exponentially growing cells. Seven cells exhibited novel nuclear DNA movements, where all, or nearly all, of the nuclear DNA passed from one side of the neck to the other (top cell in Fig. 3, B–E). In some cells, the nuclear DNA passed into the neck (bottom cell in Fig. 3 C) and then returned back to the side of the neck from which it started (bottom cell in Fig. 3 G). We refer to these movements as nuclear DNA transits. Nuclear DNA transits were not observed in either the eight wild-type cells exposed to the same temperature shift regime or the seven cdcl6-1 cells grown at 23°C. This result suggests that the nuclear DNA transits were induced by the combination of the cdcl6 mutation and the temperature shift. Several nuclear DNA transits were observed in a given cell, indicating that the DNA could move across the neck either from the mother cell to the bud or vice versa. For a typical nuclear DNA transit, the DNA moved ∼2 μm within 3–6 min. In the seven cells where nuclear DNA transits were observed, five cells subsequently entered into anaphase and completed chromosome segregation. The rate of chromosome separation in these cells (data not shown) was similar to that in exponentially growing wild-type or cdcl6-1 cells (Fig. 2). Thus, nuclear DNA transits did not preclude subsequent completion of chromosome segregation or dramatically alter the rate of chromosome movement during anaphase.

**Distribution of Nuclear DNA in Fixed Cells**

To ensure that the observed nuclear DNA movements were not induced by DIM or by the growth conditions under the coverslip, we examined the distribution of nuclear DNA between the mother cell and bud in large numbers of fixed cells stained with both calcofluor and DAPI. Calcofluor stains chitin rings found at the neck of all dividing cells (16) and additional rings, bud scars, that are found exclusively on the surface of mother cells that have undergone previous divisions. Therefore, the distribution of the nuclear DNA between the mother cell and bud was determined unambiguously in fixed cells where a bud scar was evident.

The distribution of nuclear DNA was similar for an exponentially growing population of wild-type cells, grown under three different temperature regimes, and an exponentially growing population of cdcl6-1 cells, grown at 23°C (Table I). In budded cells, nuclear DNA was found predominately on one side of the neck (58%) or segregated into both the mother cell and bud (38%) but rarely (4%) spanning the neck (Table I, part A). The doubling time for an exponentially growing population of cells is 150 min. We observed nuclear DNA passing through the neck in 2–3 min with DIM. Therefore, the fraction of cells with DNA spanning the neck should be ∼1–2% (2/150), a value consistent with the

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**References:**

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Time-lapse images of chromosome segregation in exponentially growing *S. cerevisiae* using DIM. Images are of *cdc16* cells growing at their permissive temperature, 23°C. *A* and *C* are phase images taken at the beginning (0 min) and end (70 min) of image collection, respectively. *B* and *D* are the fluorescence images of *A* and *C*, respectively. *E*–*O* are fluorescence images taken at the following time points: 0, 30, 32, 36, 40, 42, 44, 48, 52, 58, and 70 min, respectively. To view the movement of DNA relative to the periphery of the cell, a template of the periphery of the cell was made from each phase image (like those in *A* and *C*) and superimposed upon the corresponding fluorescence image. Arrows point to the nuclear DNA. *P*–*T* are the same images as *G*, *H*, *J*, *L*, and *O*, respectively; however, a reference axis (dotted line) and a segregation axis have been added. Bar, 1 μm.

**Nuclear Envelope and Spindle Structure in cdc16 Cells Recovering from Arrest**

Nuclear DNA transits in *cdc16* cells might occur by the movement of the nucleus across the neck or by the movement of chromosomes within the nucleus. We used electron microscopy to examine the morphology of the nuclear envelope in *cdc16* cells recovering from arrest. The morphology of the nuclear envelope was scored in longitudinal sections where both the mother cell and bud were of equal size to ensure that the plane of section was through the middle of the cell. The nucleus was unambiguously stretched across the neck in 26 of 27 cells examined (Fig. 5 c), suggesting that during a nuclear DNA transit chromosomes migrate across the neck within an extended nucleus.

The role of the spindle in generating nuclear DNA transits was examined in *cdc16* cells that were fixed while recovering from arrest. The position of the spindle in 100 cells was examined by indirect immunofluorescence using an anti-tubulin antibody. The spindle had three basic configurations: either it spanned the neck (Fig. 4 e), crossed the neck at an angle (Fig. 4 f), or was retained completely in the mother cell or bud (Fig. 4 g). The first two configurations were also observed in several electron micrographs (Fig. 5 b; data not shown). Therefore, in *cdc16* cells recovering from arrest, the position of the spindle was variable. In cells with all of the nuclear DNA in the mother cell, the spindle either crossed the neck directly or at an angle 90% of the time and was entirely within the mother cell the remaining 10%. Similarly, when the nuclear DNA was in the bud, the spindle spanned the neck 70% of the time and was entirely in the bud the remaining 30%. When the nuclear DNA spanned the neck, the spindle did also. The spindle and nuclear DNA were never observed in different parts of the cell. Thus, the colocalization of the spindle with the nuclear DNA is consistent with the idea that the spindle is responsible for directing DNA movement during nuclear DNA transits.

We measured the length of the spindle in cells recovering from *cdc16* arrest (see Materials and Methods). The spindle exhibited variable lengths whether it spanned the neck or was confined entirely in the mother cell or bud (Fig. 6). Spindles spanning the neck were longer (4 ± 0.1 μm) than spindles contained entirely in the mother cell or bud (2.8 ± 0.07 μm). A small amount of this variability may result from the spindle being tilted relative to the plane of focus. However, the majority of spindles were measured from images where both SPBs were in focus. Given the narrow depth of field for the 100× objective, the maximum error in spindle length resulting from focusing artifacts would be 20%, which could not account for the 200% difference in spindle lengths observed. Thus, in cells recovering from *cdc16* arrest, the length and the position of the spindle were variable.
Table I. Position of Nuclear DNA

| Strain | Temp     | On one side of neck | Through the neck | Segregated | Cells examined |
|--------|----------|---------------------|------------------|------------|----------------|
|        | °C       | %                   | %                | %          | %              |
| Wild type | 23       | 59.4                | 3.9              | 36.6       | 303            |
|         | 23/36    | 55.6                | 3.6              | 40.6       | 300            |
|         | 23/36/23 | 59.4                | 2.8              | 37.7       | 281            |
| cdc16-1 | 23       |                     |                  |            |                |
|         | 23/36    | 64.0                | 19.5             | 16.5       | 600            |
|         | 23/36/23 | 63.3                | 22.2             | 14.5       | 600            |
| cdc13-1 | 23       |                     |                  |            |                |
|         | 23/36    | 73.4                | 26.3             | 0.3        | 300            |
|         | 23/36/23 | 81.7                | 17.3             | 1.0        | 300            |
| cdc23-1 | 23       |                     |                  |            |                |
|         | 23/36    | 67.6                | 20.4             | 11.5       | 139            |
|         | 23/36/23 | 70.2                | 21.7             | 8.1        | 161            |
| cdc20-1 | 23       |                     |                  |            |                |
|         | 23/36    | 88.8                | 3.1              | 8.1        | 320            |
|         | 23/36/23 | 53.5                | 5.7              | 40.8       | 314            |

The position of the nuclear DNA was determined in fixed cells that were stained with DAPI to visualize DNA and calcofluor to visualize chitin. Cells with cdc mutations were fixed from an exponentially growing asynchronous population (23°C), from an arrested population (23/36°C), and from a population that was arrested and then allowed to recover from arrest for 30 min (23/36/23°C). Exponentially growing wild-type cells were subjected to the same temperature regimes. The nuclear DNA was located in three positions in the cell: on one side of the neck; through the neck between the mother cell and the bud; or segregated, with one genome located in the mother cell and the other in the bud (part A). In the cells where the nuclear DNA was on one side of the neck, we determined if the DNA was in the mother cell or the bud (see Materials and Methods) (part B).

which suggests that the spindle may exhibit dynamic movements within the elongated nucleus.

Nuclear DNA Transits in Other cdc Strains

To determine whether the induction of nuclear DNA transits was cdc16 specific, the position of nuclear DNA in other cdc mutants was examined. We initially analyzed cdc13, cdc20, and cdc23 mutants because they arrest with the same morphology as cdc16 mutants (24). Cells were fixed while growing exponentially (23°C), while arrested (23/36°C), or while recovering from arrest (23/36/23°C), and the position of the nuclear DNA in the mother cell and bud was determined by staining with both calcofluor and DAPI (Materials and Methods).

The nuclear DNA morphology in cdc13-1 or cdc23-1 cells was similar to what had been observed in cdc16-1 cells (Table I, part B). Exponentially growing cultures, independent of the cdc mutation, had a small fraction of budded cells with the nuclear DNA entirely in the bud (%). However, like cdc16-1 cells, >50% of cdc13-1 and cdc23-1 cells that were arrested or recovering from arrest had all the nuclear DNA in the bud (Table I, part B). These results suggest that nuclear DNA transits are enhanced when cells with these mutations are arrested or allowed to recover from arrest. In addition, ~20% of these cells had nuclear DNA through the neck (Table I, part A). As suggested above, the abundance of cells in this class may reflect the frequent passage of DNA into the neck during nuclear DNA transits.

On the other hand, cdc20-1 cells that were arrested or recovering from arrest had significantly different distributions of nuclear DNA than cdc13, 16, or 23 cells. The fraction of cdc20-1 cells with all or nearly all of the nuclear DNA in the bud was only 4% in exponentially growing cells and did not increase in arrested cells or cells allowed to recover from arrest (Table I, part B). Furthermore, the fraction of cells with nuclear DNA through the neck did not increase (Table I, part A). These results suggest that arresting cells with this cdc20 mutation does not induce nuclear DNA transits.

Discussion

We used DIM to examine chromosome movements in live yeast cells stained with the DNA-specific fluorescent dye, DAPI. This technique enabled us to determine the rate of chromosome separation and to observe subtle features of chromosome movement during anaphase, including changes

Figure 3. Time-lapse images of chromosome segregation in cells recovering from cdc16 arrest using DIM. cdc16-1 cells were grown at 23°C, shifted to 36°C for 3 h to arrest cells, and then returned to 23°C to release cells from arrest. A is a phase image of the cells taken at the end of the observation period. Phase images taken at the beginning or at intermediate points (not shown) were indistinguishable. B—I are fluorescence images taken at the following time points: 0, 3, 12, 18, 36, 39, 40, and 70 min, respectively. To view the movement of DNA relative to the periphery of the cell, a template of the periphery of the cell was made from the phase image in A and superimposed upon the fluorescence images. Arrows point to the nuclear DNA. Bar, 1 μm.
Fluorescence images of cdcl6-1 cells grown at 23°C, shifted to their restrictive temperature, 36°C, for 3 h, and then released from the arrest by returning them to 23°C for 15 min. Cells in a–d were fixed and stained with DAPI to visualize the nuclear DNA (black arrowheads) and calcofluor to visualize chitin (white arrows). Cells were observed with either the nuclear DNA passing through the isthmus, or neck, between the mother cell and bud (a); being retained in the mother cell, distinguished by chitin bud scars present on the cell surface (b); or being retained completely, or nearly completely, in the bud, determined by the absence of a bud scar (c and d). Cells in e–g are indirect immunofluorescence images of tubulin structures stained with anti-tubulin antibodies as described in Materials and Methods. The spindle is the structure observed between the SPBs, which are discernable in each cell as two brightly stained regions with cytoplasmic microtubules radiating out to the cell surface. The spindle was observed spanning the neck from mother cell to bud (e), spanning the neck at an angle (f), or located completely in the mother cell or bud (g). Bar, 1 μm.

Chromosome Movement in Exponentially Growing Cells
The kinetics of chromosome movement in exponentially growing yeast cells revealed several striking similarities to mitosis in higher eukaryotes. The rate of separation of the yeast genomes, 1 μm/min, is similar to that (1–3 μm/min) in PtK kangaroo rat cells (26) and in newt lung epithelial cells (NLC) (4). From our preliminary analysis, sister genomes did not separate from each other at a continuous rate; rather, several pauses appeared to occur. It is interesting to note that discontinuous movement of chromosomes has also been suggested in anaphase of NLC cells (4, 25).

During anaphase in yeast, the orientation of the segregation axis (a line between segregating genomes) changed relative to an axis through the neck. Several lines of evidence suggest that changes in the orientation of the segregation axis reflect changes in the orientation of the spindle. In exponentially growing cultures, the spindle of some cells is oriented in positions that are similar to the locations of the segregation axis (Palmer, R. E., and D. Koshland, unpublished results). In addition, the rotation of the spindle has been observed during prometaphase in NLC cells (4), during metaphase in other fungi (1, 2), and during telophase in guard mother cells of Allium cepa (22). Interestingly, these changes in the orientation of the yeast spindle during anaphase may not be random. In the 70% of cells where the orientation of the segregation axis changed, the axis (and presumably the spindle) always rotated during or just after the DNA crossed the neck. This specific reorientation of the segregation axis may not have been observed in 30% of the cells because in these cells rotation occurred in a plane perpendicular to the plane of focus. Programmed reorientation of spindles is a common feature of mitosis in other fungi (1), stomatal differentiation in Allium cepa (22), and embryogenesis in Caenorhabditis elegans (18).

Morphological differences between yeast and higher eukaryotic cells during mitosis suggest that some aspects of mitosis differ between these cell types. For example, during mitosis in yeast, the nuclear envelope does not breakdown, and the spindle has very few kinetochore or pole-to-pole microtubules (23). However, recent experiments suggest that a high degree of structural and functional similarity exists between the maturation-promoting factor of Xenopus laevis, the cdc2 gene product of Schizosaccharomyces pombe, and the CDC28 gene product of Saccharomyces cerevisiae. This fact suggests that molecules that regulate the cell cycle...
Figure 5. Electron micrographs ofcdc16-1cells that were shifted to their restrictive temperature, 36°C, for 3 h to arrest them and then released from their arrest by returning the cells to 23°C for 15 min. a and b are different cells where the nucleus (n) is stretched across the neck between the mother cell and bud. c is an enlargement of b. Note invagination (l) of the nuclear envelope where the SPB is attached to the nuclear envelope. The spindle (S) almost completely crosses the nucleus. Bar: (a and b) 1 μm; (c) 3.5 μm.
are highly conserved (3, 5, 6, 10-12). Furthermore, from the data presented in this study, the three most striking observations of yeast anaphase are the absolute rate of chromosome separation, the pauses in chromosome separation, and the apparent rocking of the spindle. All of these features have precedents in anaphase of higher eukaryotes, suggesting that the mechanism of chromosome movement during anaphase may be conserved between yeast and higher eukaryotes.

**Nuclear DNA Transits**

We observed nuclear DNA transits in cells recovering from cdc16-1 arrest. Any hypothesis on the mechanism of these transits must take into account the morphological features of cells undergoing these transits, including the presence of an elongated nucleus extending into both the mother cell and bud, variability in the length and position of the spindle, and colocalization of the nuclear DNA with the spindle. Since the nucleus is elongated between the mother cell and bud, nuclear DNA transits are occurring by a mechanism that moves chromosomes within the nucleus. It is reasonable to suggest that the spindle directs chromosome movement during nuclear DNA transits given the observed colocalization of the spindle and chromosomes and the apparent role of the spindle in moving chromosomes within the nucleus (17). We propose two models to explain how the position of the spindle and the associated chromosomes may change (Fig. 7). In model I, the spindles may undergo a series of asymmetric expansions and contractions that cause them to move to a new position within the nucleus. Changes in spindle microtubule lengths have been associated with oscillatory movement of individual chromosomes during prometaphase/metaphase in higher eukaryotes (4, 25). Alternatively (model II), the spindles may have fixed lengths and their positions in the nucleus may change by extranu-
clear forces, perhaps exerted through cytoplasmic microtubules that emanate from the SPBs. This model has been proposed for prometaphase/metaphase nuclear DNA movements in other fungi (2). We prefer model I because it explains the observed variability in spindle lengths; however, we cannot exclude the possibility that cdc16 cells arrest with variable spindle lengths that remain fixed until the onset of anaphase.

The position of nuclear DNA in fixed cells indicated that nuclear DNA transits are rarely seen in wild-type cells but are enhanced in cdc13, cdc23, and cdc16 strains that were arrested or allowed to recover from arrest. It is possible that these mutants all induce transits because they affect proteins directly required for proper chromosome movement. In fact, the products of CDC6 and CDC23 share amino acid homology and may be functionally related (Sikorski, R., and P. Heiter, personal communication). However, genetic analysis of the CDC13 gene suggests that its product may be required for DNA metabolism (Weinert, T., and L. Hartwell, personal communication). Furthermore, nuclear DNA transits occur in cdc17 cells (Palmer, R. E., and D. Koshland, unpublished results) that have a defect in DNA polymerase (9). Therefore, it seems unlikely that all these mutants induce nuclear DNA transits because they are defective in some component of the segregation apparatus.

As an alternative model, nuclear DNA transits in some of these mutants may result from the oscillation of chromosomes that occur normally during prometaphase and metaphase. In fact, in other eukaryotes, chromosome oscillation has been observed during these stages of mitosis (1, 2, 4, 25). We suggest two explanations why these oscillations frequently give rise to nuclear DNA transits in cells arrested before anaphase but infrequently in exponentially growing cells. One possibility is that prometaphase and metaphase are sufficiently short in exponentially growing cells that these cells usually enter anaphase before an oscillation results in a nuclear DNA transit. On the other hand, cells arrested before anaphase have ample time to complete transits. Alternatively, the magnitude of chromosome oscillation in exponentially growing cells may be insufficient to cause a transit. However, prolonged arrest of cells before anaphase may cause the magnitude to become sufficiently exaggerated to generate nuclear DNA transits.

Cells arrested by a cdc20 mutation fail to undergo nuclear DNA transits. In addition, cells arrested with the microtubule-depolymerizing drug, nocodazole, also fail to undergo nuclear DNA transits (our unpublished results and reference 20). The failure to observe nuclear DNA transits in nocodazole-treated cells is not surprising since the nucleus cannot migrate to the neck and, thus, remains entirely in the mother cell (20). The failure to observe nuclear DNA transits in a cdc20 mutant is more interesting since this mutant, like cdc16, cdc13, and cdc23, arrests before the onset of anaphase with the nucleus spanning the neck and a short spindle (8). In addition, our preliminary results suggest that cells released from cdc20 arrest appear to commence chromosome separation immediately, suggesting a direct involvement between the CDC20 gene product and the movement of chromosomes during anaphase. One possibility is that the cdc20 cells may arrest at a stage in the cell cycle after nuclear DNA transits can take place but before the onset of anaphase. Alternatively, the CDC20 gene product may be directly required for chromosome movement: e.g., a component of the spindle that directs proper assembly of microtubules, a component of the kinetochore necessary for microtubule binding, or a component of a mitotic motor. To address if the gene product has any of these functions, we are analyzing other mutant alleles of the CDC20 gene as well as cloning the wild-type gene.

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