MOLECULAR AND CELLULAR BIOLOGY, Dec. 1995, p. 6572–6581 Vol. 15, No. 12
0270-7306/95/$04.00+0

NDT80, a Meiosis-Specific Gene Required for Exit from Pachytene in *Saccharomyces cerevisiae*

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Received 30 May 1995/Returned for modification 31 July 1995/Accepted 5 September 1995

We describe the identification of a new meiosis-specific gene of *Saccharomyces cerevisiae*, *NDT80*. The *ndt80* null and point mutants arrest at the pachytene stage of meiosis, with homologs connected by full-length synaptonemal complexes and spindle pole bodies duplicated but unseparated. Meiotic recombination in an *ndt80*Δ mutant is relatively normal, although commitment to heteroallelic recombination is elevated two- to threefold and crossing over is decreased twofold compared with those of the wild type. *ndt80* arrest is not alleviated by mutations in early recombination genes, e.g., *SPO11* or *RAD50*, and thus cannot be attributed to an intermediate block in prophase chromosome metabolism like that observed in several other mutants. The *ndt80* mutant phenotype during meiosis most closely resembles that of a *cdc28* mutant, which contains a thermolabile p34, the catalytic subunit of maturation-promoting factor. Cloning and molecular analysis reveal that the *NDT80* gene maps on the right arm of chromosome VIII between *EPT1* and a Phe-tRNA gene, encodes a 627-amino-acid protein which exhibits no significant homology to other known proteins, and is transcribed specifically during middle meiotic prophase. The *NDT80* gene product could be a component of the cell cycle regulatory machinery involved in the transition out of pachytene, a participant in an unknown aspect of meiosis sensed by a pachytene checkpoint, or a *SPO11*- and *RAD50*-independent component of meiotic chromosomes that is the target of cell cycle signaling.

During meiosis, two rounds of chromosome segregation follow a single round of DNA replication. In the first meiotic division, pairs of sister chromatids (homologs) disjoin; at the second meiotic division, sister chromatids segregate to opposite poles as in mitosis.

Meiotic prophase, the period between DNA replication and the first nuclear division, involves a complex series of changes in the chromosomes, which include association of sister chromatids, organization of each homolog into a linear array of loops connected at their bases by an axial or lateral element, and interactions between homologs. Interhomolog interactions, in turn, comprise three aspects: pairing, recombination, and formation of synaptonemal complexes (SCs). Pairing and recombination appear to be two different manifestations of a single series of events occurring at a number of interstitial locations along the chromosomes (37, 74). At early stages in this process, homologs become coaligned along their lengths; the first chemical steps of recombination, the formation of meiosis-specific double-strand breaks (DSBs), also occur during these early stages (50, 51). At intermediate stages, the coaligned homolog axes become intimately connected via a prominent structure, the SC; at about this same time, some or all DSBs are converted to double Holliday junctions (58, 59).

At the next stage of meiotic prophase, pachytene, SCs occur along the entire lengths of all homolog axes. The recombination process is completed at about the end of pachytene; mature crossover and noncrossover recombination products both occur at about this time (51, 68). At pachytene, chromatin loops are tightly condensed (16). Another diagnostic feature of pachytene in *Saccharomyces cerevisiae* is that spindle pole bodies (SPBs) are duplicated but not yet separated, with mother and daughter SPBs lying side by side within the nucleus (11). Exit from pachytene is signaled by the disassembly of SCs and the reorganization of the chromosome structure in preparation for the first nuclear division. In organisms with large chromosomes, dramatic compaction can occur at this stage, but in *S. cerevisiae* no further compaction is apparent (16, 50). SPBs also separate following SC disassembly (51) to form a short spindle that corresponds approximately to metaphase I (48, 52).

The transition from pachytene to metaphase I is an important point for regulation of meiotic development in many organisms. Meiosis in some organisms involves a very extended arrest at the end of prophase (see, e.g., reference 41). In *S. cerevisiae*, exit from pachytene is a crucial regulatory point. A mutation in the gene encoding the catalytic subunit of maturation-promoting factor (MPF), *CDC28*, causes meiotic cells to arrest at pachytene (62). A mutation in the gene for the regulatory subunit of MPF, *CLB1*, also causes arrest prior to meiosis I, presumably at pachytene (23). Pachytene arrest is also conferred by mutations in two genes, *CDC36* and *CDC39*, which resemble *CDC28* in that mutations in them confer arrest at the G_1 stage during the mitotic cell cycle (62). Shuster and Byers (62) suggest that pachytene serves as a cell cycle regulatory checkpoint and that this stage is crucial because it represents the last point at which *S. cerevisiae* can return to the mitotic cell cycle without having undergone a reductional division.

Pachytene also appears to be an important meiotic cell cycle checkpoint. Meiotic cells monitor the progression of chromosome metabolism and arrest at pachytene in response to certain critical defects, presumably via changes in MPF activity. In particular, mutations in a number of genes cause intermediate blocks in the meiotic recombination pathway and lead to cell cycle arrest. The first genes identified in this category were *DMC1*, encoding a meiosis-specific RecA homolog (9), and *RAD50*, with which arrest is conferred by certain meiosis-specific alleles (*rad50S* [4]). Other genes in this category include four newly isolated intermediate-block genes, *SAE1*, *SAE2*,

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TABLE 1. Experimental strains used

Strain	Genotype
NKY611	**a**/α *ho*::*LYS2*/*ho*::*LYS2 lys2*/*lys2 ura3*/*ura3 leu2*::*hisG*/*leu2*::*hisG*
NKY895	**a**/α *HO*/*HO ste7-1*/*ste7-1 ade2*/*ade2 lys2*/*lys2 ura3*/*ura3 leu2*::*hisG*/*leu2*::*hisG*
NKY1276	**a**/α *HO*/*HO ste7-1*/*ste7-1 ade2*/*ade2 lys2*/*lys2 ura3*/*ura3 leu2*::*hisG*/*leu2*::*hisG spo13*Δ::*LEU2*/*SPO13 spo11*Δ::*hisG-URA3-hisG*/*spo11*Δ::*hisG-URA3-hisG* with pNKY1159
NKY1063	α *ho*::*LYS2 ade2 lys2 ura3 leu2*::*hisG*
NKY2172	**a**/α *HO*/*HO ste7-1*/*ste7-1 ade2*/*ade2 lys2*/*lys2 ura3*/*ura3 leu2*::*hisG*/*leu2*::*hisG ndt80-1*/*ndt80-1*
NKY2205	**a**/α *ho*::*LYS2*/*ho*::*LYS2 lys2*/*lys2 ura3*/*ura3 leu2*::*hisG*/*leu2*::*hisG ndt80*Δ::*LEU2*/*NDT80*
NKY2296	**a**/α *ho*::*LYS2*/*ho*::*LYS2 lys2*/*lys2 ura3*/*ura3 leu2*::*hisG*/*leu2*::*hisG ndt80*Δ::*LEU2*/*ndt80*Δ::*LEU2*
NKY2534	**a**/α *ho*::*LYS2*/*ho*::*LYS2 lys2*/*lys2 ura3*/*ura3 leu2*::*hisG*/*leu2*::*hisG spo11*Δ::*hisG*/*spo11*Δ::*hisG*
NKY2535	**a**/α *ho*::*LYS2*/*ho*::*LYS2 lys2*/*lys2 ura3*/*ura3 leu2*::*hisG*/*leu2*::*hisG spo11*Δ::*hisG*/*spo11*Δ::*hisG ndt80*Δ::*LEU2*/*ndt80*Δ::*LEU2*
NKY2536	**a**/α *ho*::*LYS2*/*ho*::*LYS2 lys2*/*lys2 ura3*/*ura3 leu2*::*hisG*/*leu2*::*hisG rad50*Δ::*hisG*/*rad50*Δ::*hisG*
NKY2537	**a**/α *ho*::*LYS2*/*ho*::*LYS2 lys2*/*lys2 ura3*/*ura3 leu2*::*hisG*/*leu2*::*hisG rad50*Δ::*hisG*/*rad50*Δ::*hisG ndt80*Δ::*LEU2*/*ndt80*Δ::*LEU2*
NKY2201	**a**/α *ho*::*LYS2*/*ho*::*LYS2 lys2*/*lys2 ura3*/*ura3 leu2*::*hisG*/*leu2*::*hisG his4XLEU2-Mlu*I-*URA3*/*his4BLEU2-Mlu*I
NKY2310	**a**/α *ho*::*LYS2*/*ho*::*LYS2 lys2*/*lys2 ura3*/*ura3 leu2*::*hisG*/*leu2*::*hisG ndt80*Δ::*LEU2*/*ndt80*Δ::*LEU2 his4XLEU2-Mlu*I-*URA3*/*his4BLEU2-Mlu*I
NKY1464	**a**/α *ho*::*LYS2*/*ho*::*LYS2 lys2*/*lys2 ura3*/*ura3 leu2*::*hisG*/*leu2*::*hisG arg4-Bgl*/*arg4-Nsp*
NKY2647	**a**/α *ho*::*LYS2*/*ho*::*LYS2 lys2*/*lys2 ura3*/*ura3 leu2*::*hisG*/*leu2*::*hisG arg4-Bgl*/*arg4-Nsp ndt80*Δ::*LEU2*/*ndt80*Δ::*LEU2*

and *SAE3* (43) and *MEI5* (47). Mutations in all of these genes except *MEI5* also cause either a delay or a defect in the formation of SCs. In addition, mutations in the *ZIP1* gene, which encodes an important component of the SC central region, causes recombination defects and arrest at the end of prophase (71). In all of these cases, the mutant arrest phenotype is eliminated by mutations that block the meiotic chromosome metabolism pathway at a very early stage, e.g., mutations in *SPO11* (36), *RAD50* (4), or *MEI4* (45). Presumably an upstream block prevents the process from ever reaching the critical intermediate block. Arrest in these mutants has been attributed variously to defects in the progression of interhomolog interaction complexes (77a) and to defects in the formation of the SC (71).

Arrest at the end of meiotic prophase, prior to SPB separation, is also conferred by mutations in three other genes, *SPO15* (80), *TOP2* (53), and *SEP1* (6, 72, 73). None of these mutations yet falls cleanly into the general category described above.

(i) The *SPO15* gene encodes a dynamin-like protein (81), and *spo15* arrest is alleviated by a mutation in the *RAD50* gene (79); it has been proposed that a defect in spindle development leads to a defect in chromosome metabolism.

(ii) *TOP2* encodes DNA topoisomerase II. *top2* arrest is also alleviated by a *rad50* mutation, but both recombination and SC formation in a *top2* mutant are normal (53). In this case, arrest may be triggered by some unidentified defect in chromosome metabolism; alternatively, topoisomerase II might be a structural component of meiotic chromosomes whose activity is required directly for SC disassembly, which in turn may be required for SPB separation.

Existing observations are consistent with and suggestive of a dependency of SPB separation upon SC disassembly. Most strikingly, in wild-type SK1 meiosis, nuclei that contain partially disassembled SCs always have SPBs unseparated, while nuclei that contain separated SPBs never contain any trace of either tripartite SC structure or axial elements (48, 51). It seems improbable that such complete separation of the two events would be observed without some specific dependence of the latter event on completion of the former.

(iii) *SEP1* is involved in many mitotic processes, including mRNA turnover and rRNA processing in mitotic cells (27, 31, 39, 49, 66, 67); recent observations suggest a role of *SEP1* in microtubule function (32). A *sep1* mutation causes meiotic chromosome metabolism defects (6, 73), but arrest is not alleviated by a *rad50* mutation (72). Perhaps more than one defect confers arrest in this case.

Pachytene may also be an important control point when the meiotic process is interrupted by exposure to nutrients. Yeast cells undergo meiosis and sporulation in response to starvation for critical nutrients, but this process can be interrupted by the reintroduction of appropriate sources of carbon and nitrogen. In such a "return-to-growth" regimen, cells that are in stages up to and including pachytene can return to a mitotic cell cycle without carrying out two meiotic nuclear divisions and spore formation, whereas cells that have completed meiosis I also complete meiosis II and spore formation before initiating a mitotic cycle (17, 29, 61, 62). Cells are capable of returning to the mitotic cycle at later stages only if meiosis is arrested in some way, and even then resumption of the mitotic cycle takes much longer than it does in cells at or prior to pachytene (30).

Finally, it is possible that pachytene represents a checkpoint for processes other than chromosome metabolism (see Discussion).

We describe below the identification and characterization of a new meiosis-specific gene, *NDT80*, which is required for exit from the pachytene stage.

MATERIALS AND METHODS

Strains. *Escherichia coli* MM294 (F^- *endA hsdR supE44 thiA*) was used for all plasmid manipulations (24). All yeast strains are derivatives of SK1 (34, 75) and are listed in Table 1. Markers of yeast strains include *ho*::*LYS2*, *lys2*, *ura3*, *leu2*::*hisG*, *arg4-Bgl*, *arg4-Nsp*, *ste7-1*, *ade2*, *ndt80-1*, *ndt80*Δ::*LEU2*, *spo11*Δ::*hisG-URA3-hisG*, *spo11*Δ::*hisG*, *rad50*Δ::*hisG*, *spo13*Δ::*LEU2*, *his4XLEU2-Mlu*I-*URA3*, and *his4BLEU2-Mlu*I (3, 4, 12, 21, 43; this work).

Media. Media for growth of yeast strains were prepared as described previously (60). YPD contained 1% yeast extract, 2% Bacto Peptone, and 2% glucose. YPA was 1% yeast extract, 2% Bacto Peptone, and 1% potassium acetate. Synthetic complete medium consisted of 0.67% yeast nitrogen base without amino acids (Difco), 0.09% amino acid powder, and 2% glucose. The components of the amino acid powder were as described by Bishop et al. (9). Dropout media are synthetic complete medium that lacks a specific amino acid(s). Regular medium for sporulation (SPM) contained 0.3% potassium acetate and 0.02% raffinose. For plates, 2% Bacto agar was added. Rich SPM plates contained 0.25% yeast extract, 2% potassium acetate, 0.09% dropout powder, 0.1% glucose, and 2% Bacto agar.

Plasmids. pNKY1159 is a centromere-containing plasmid carrying the *ADE2* and *SPO11* genes (43). It can replicate in yeast cells because of the *ARS* function provided by sequences in *ADE2* and *SPO11* (5, 56). pYe511 is a 3.0-kb *Hin*dIII fragment of *ARG4* cloned into pBR322 (7). pNKY155 and pNKY291 contain the

HIS4 sequence and sequence downstream of *HIS4*, respectively, and have been described previously (12, 77). pNKY422 is a pBluescriptII derivative harboring a 0.9-kb *DMC1* cDNA insert (9). Plasmids pNKY1210 and pNKY1211 are the original *NDT80*-complementing plasmids isolated from a YCp50-based genomic library (54). Other plasmids were constructed by standard procedures (55). Subcloning of a 4.3-kb *Cla*I fragment derived from the insert of pNKY1210 into pRS316 (63) resulted in pNKY1212. pNKY1213 is the smallest nested deletion subclone of pNKY1212 that still complements the *ndt80-1* mutation. In pNKY1214, a 2.7-kb *Xho*I-*Bgl*II fragment of *LEU2* from YEp13 replaced the 1.2-kb *Eco*47III-*Bam*HI fragment of pNKY1213.

Isolation of mutants. Mutants were obtained by using a newly developed approach for identification of recessive meiotic mutants (42). This approach utilizes a homothallic strain bearing a *ste7-1* mutation, which blocks mating at nonpermissive temperatures (25). A culture of such a strain (NKY1276) was mutagenized with 3% ethylmethane sulfonate, mutagenized cells were taken through meiosis, and the resulting individual spores were plated and germinated on growth medium at 34°C, a temperature nonpermissive for the *ste7-1* mutation. Under these conditions, the resulting single colonies contain haploid cells of both mating types. These colonies were then treated in parallel in two ways. (i) To identify mutants, cells from such colonies were patched onto a growth plate and incubated at 18°C, a permissive temperature to permit diploidization. These grown patches were then replica plated onto a nitrocellulose filter on a sporulation plate, the plates were incubated to permit sporulation, and patches were examined for spore formation by dityrosine fluorescence (10). Mutations of interest were examined further (see Results). (ii) Mutations of interest were recovered into heterozygous diploid strains by patching a second aliquot of cells from the original colony onto a plate seeded with an appropriately marked heterothallic haploid strain (NKY1063). After incubation at a permissive temperature, the desired heterozygous diploids form and can be selected with appropriate markers. Recessive mutations in such diploids can be subjected to standard genetic analysis to determine whether the phenotype of interest exhibits 2:2 segregation as expected for a trait resulting from a single mutation.

Other genetic procedures. Yeast transformations were carried out by electroporation (8). To disrupt the *NDT80* locus, plasmid pNKY1214 was cut with *Bgl*II and the 3.8-kb fragment was targeted for substitution. Substitutive transformations were verified by Southern blot analysis (65). Cells were assayed for sensitivity to methylmethane sulfonate according to the procedure of Bishop et al. (9). Return-to-growth experiments were performed by methods described previously (17, 61).

Cytology. The occurrence of meiotic divisions and spore formation was monitored by staining with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) and fluorescence and/or phase-contrast microscopy. Meiotic cells were taken at various times after initiation of meiosis and fixed with 50% ethanol before being stained with 1 μg of DAPI per ml. To visualize SCs by light or electron microscopy, cells were lysed, spread, and stained with silver nitrate according to the method of Loidl et al. (40). Tubulin staining of yeast cells was performed as described previously (4). For electron microscopy, cells were examined with a JEOL 100 CXII electron microscope set at 40 kV and a 4,800× magnification. For light microscopy, cells were visualized with a Zeiss Axiophot immunofluorescence microscope.

DNA sequence analysis. Nested deletion subclones were generated from pNKY1212 by using exonuclease III (26). Dideoxy sequencing reactions were carried out with ^{32}P-labeled universal T3 and T7 primers (New England Biolabs) and *Taq* DNA polymerase by using the double-stranded DNA cycle sequencing system (Gibco BRL).

Other nucleic acid techniques. Cells were taken through synchronous meiosis as described previously (12), and aliquots were collected at various times during meiosis. DNA was extracted (9, 12), digested with the appropriate restriction enzyme, fractionated on a 0.8% agarose gel in TAE buffer, and transferred onto a nylon membrane (Hybond N+; Amersham) (55, 65). RNA was isolated by a method described by McKinney et al. (44). About 10 μg of total RNA was fractionated on a 1% formaldehyde agarose gel. RNA was blotted onto a nylon membrane (55). The probe DNAs used for Southern and Northern (RNA) analyses were derived from pNKY155, pNKY291, pNKY1212, pYe511, and pNKY422. ^{32}P-labeled probes were prepared by the random priming method (18). Hybridization was carried out as described by Church and Gilbert (14).

Nucleotide sequence accession number. The GenBank accession number for the sequence of *NDT80* is U35122.

RESULTS

Identification of *ndt80-1*. The *ndt80-1* (for non dityrosine) mutation was identified in a general screen for mutants defective in spore formation as assayed by dityrosine fluorescence (see Materials and Methods). Mutant candidates were screened initially to determine whether the spore formation defect was alleviated by elimination of the *SPO11* gene, as is known to be the cases for a number of mutations that block meiotic chromosome metabolism at an intermediate stage (see the introduction).

TABLE 2. Sporulation and spore viability[a]

Strain	Relevant genotype	Sporulation (%)	Spore viability (%)
NKY895	*NDT80/NDT80*	85	95
NKY2172	*ndt80-1/ndt80-1*	<0.5	
NKY2205	*NDT80/ndt80*Δ::*LEU2*	87	95
NKY2296	*ndt80*Δ::*LEU2/ndt80*Δ::*LEU2*	<0.5	

[a] Yeast strains were grown on YPD plates and then patched on SPM plates and incubated at 30°C for 24 to 36 h. Spore formation was examined by phase-contrast microscopy. Spore viability was determined by dissecting 10 tetrads from each diploid strain.

A total of 25 mutants whose arrest were not *SPO11* dependent and for which the responsible defect exhibited 2:2 segregation were identified (2). Screening of these 25 mutants for the occurrence of meiotic nuclear divisions, the formation of SCs and meiotic spindles, and spore formation revealed a single mutant, the *ndt80-1* mutant, that is completely defective in producing ascospores (Table 2) and arrests at the pachytene stage with full-length SCs and with SPBs duplicated but unseparated, as described in detail below.

Cloning of *NDT80*. The *NDT80* gene was cloned from a YCp50-based yeast genomic library (54) by complementation of the *ndt80-1* sporulation defect. Pools of library DNA were transformed into an *ndt80-1* mutant strain (NKY2172). Ura$^+$ transformants were selected and replica plated onto sporulation medium containing a trace of nutrients (rich SPM plates; see Materials and Methods). After 5 to 7 days of incubation at 30°C to permit sporulation, colonies that had given rise to spores were identified by their ability to give blue fluorescence when illuminated with long-wavelength UV light, indicating the presence of spore walls containing dityrosine (10). The presence of spores in these colonies was confirmed by light microscopy, and plasmid DNA was recovered from the corresponding colonies on the original transformation plates.

Three transformants proficient for sporulation were identified. These transformants yielded two complementing plasmids, pNKY1210 and pNKY1211, carrying inserts of about 12 and 15 kb, respectively. These two inserts overlap by about 9 kb. Subcloning localized the complementing region to a 4.3-kb *Cla*I fragment (Fig. 1). Neither of two component *Xho*I-*Cla*I fragments (1.9 and 2.4 kb; Fig. 1) can complement the *ndt80-1* mutation, indicating that the *Xho*I site lies within the gene of

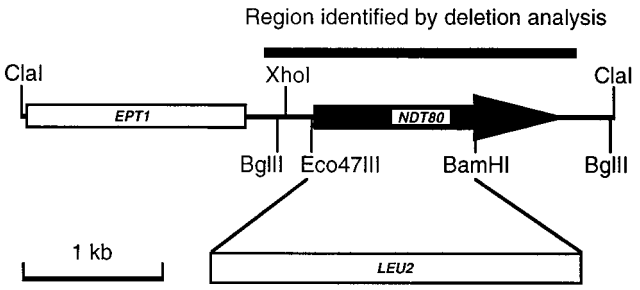

FIG. 1. Restriction map and *LEU2* disruption of the *NDT80* locus. Shown is a 4.3-kb *Cla*I fragment which complements the *ndt80-1* mutation when present on an *ARS-CEN* plasmid. The arrow depicts the direction and extent of the *NDT80* coding region. The boundaries of *NDT80* were identified by deletion-complementation analysis. The *EPT1* gene was located by sequence analysis of the region 5′ to *NDT80* together with published *EPT1* sequence information (28). In the *ndt80*Δ::*LEU2* allele, a 1.2-kb *Eco*47III-*Bam*HI fragment of *NDT80* was replaced by a 2.7-kb *Xho*I-*Bgl*II fragment of the *LEU2* gene from YEp13.

interest. Two sets of nested deletions (26) of the original 4.3-kb *Cla*I segment were made and tested for their effects on complementing activity. This analysis narrowed the region containing the complementing gene to a segment of about 2.3 kb (Fig. 1).

Disruption of the *NDT80* gene. A deletion-disruption allele of *NDT80* was constructed. The 1.2-kb *Eco*47III-*Bam*HI fragment of *NDT80* was replaced with a 2.7-kb *LEU2* fragment from YEp13 (Fig. 1). In this construct, two-thirds of the *NDT80* coding region has been deleted, and the resulting allele thus likely represents a null mutation. The *ndt80*Δ::*LEU2* allele was introduced into a wild-type diploid strain (NKY611) by substitutive transformation. The resulting strain (NKY2205), which was heterozygous for *ndt80*Δ::*LEU2*, was sporulation proficient, and all four spores of each tetrad ascus, two Leu^- and two Leu^+, were viable (Table 2). An *ndt80*Δ::*LEU2*/*ndt80-1* heterozygous diploid, made by mating the two corresponding haploid strains, is sporulation defective (data not shown). Thus, the gene cloned is bona fide *NDT80* and not a suppressor gene.

***NDT80* encodes a novel protein.** DNA sequence analysis shows that *NDT80* comprises a single 1,881-bp open reading frame encoding a putative protein of 627 amino acids (Fig. 2). The *NDT80* amino acid sequence was compared with sequences available in the GenBank database. No significant homology was found between the *NDT80* gene product and other known proteins. The protein encoded by *NDT80* exhibits several notable features, as follows.

(i) The protein is rich in charged amino acids, including both positively (7.3% [46 of 627] lysine and 5.7% [36 of 627] arginine) and negatively (8.5% [53 of 627] aspartic acid and 5.6% [35 of 627] glutamic acid) charged amino acids.

(ii) The protein contains 11.2% (70 of 627) serine and 4.9% (31 of 627) threonine. These high levels of serine and threonine residues raise the possibility that the Ndt80 protein might be subject to phosphorylation by certain protein kinases. However, no sequence within the protein has been found to match the consensus sequence for the targets of any of several protein kinases, including cyclic AMP-dependent protein kinase, protein kinase C, and cyclin-dependent kinase $p34^{CDC28}$ (19, 22, 35, 38).

(iii) The protein contains the sequence K-R-D-K (positions 176 to 179), which conforms to the consensus sequence of nuclear location signals in mammalian cells (K-R/K-X-R/K [13]). It is not known whether the requirements for nuclear localization are the same in yeast cells and mammalian cells, however.

Mapping of *NDT80*. The sequence upstream of the *NDT80* gene, positions −365 to −182, was found to be identical to the sequence downstream of the *EPT1* gene (28). Recently, the DNA sequence of the whole chromosome VIII has been reported (33), and *NDT80* is located on the right arm of chromosome VIII, between *EPT1* and a Phe-tRNA gene.

Cytological analysis of *ndt80* mutants. The *ndt80* phenotype has been analyzed most extensively for the *ndt80* null mutant (Fig. 3). Meiotic cells of isogenic wild-type and *ndt80*Δ::*LEU2* strains were taken through a meiotic time course (see Materials and Methods). The occurrence of meiotic divisions was analyzed by examining DAPI-stained cells by fluorescence light microscopy (Fig. 3A). For the wild-type strain, 90% of cells had completed meiosis after incubation in SPM for 10 h; for the *ndt80*Δ strain, in contrast, fewer than 0.5% of cells exhibited any meiotic division(s).

The formation of SCs was monitored by examining silver-stained spread nuclei by phase-contrast microscopy (Fig. 3B and C). In the wild-type strain, SCs were observed transiently, at 4 and 5 h of meiosis, and no SC was observed at time points later than 6 h. In the *ndt80*Δ mutant, in contrast, SCs appeared at the same time as in a wild-type strain (4 h) but then continued to accumulate. By 10 h, about 90% of the *ndt80*Δ cells contained SCs (Fig. 3C). The SCs persisted at these high levels in the *ndt80*Δ cells for at least 15 h, i.e., as long as it takes for wild-type cells to complete spore formation (data not shown).

The absence of SPB separation in arrested *ndt80-1* mutant cells has been demonstrated by an antitubulin immunofluorescence assay. Each arrested cell contains a single tubulin-staining focus having an "umbrella" or "bush" morphology (data not shown). This morphology is characteristic of cells held aberrantly long at the pachytene stage and results from hyperproliferation of microtubules at the site of duplicated and unseparated SPBs (1). Electron microscopic images of these structures in arrested *dmc1*Δ cells were presented by Bishop et al. (9). For technical reasons, SPBs are not visible by light microscopy in *ndt80*Δ nuclei prepared for examining SCs.

The *ndt80* pachytene arrest phenotype has been confirmed by electron microscopic examination of silver-stained spread nuclei (Fig. 4). In an *ndt80*Δ strain, at 6 h of meiosis, essentially every nucleus (26 of 26 nuclei examined) contained full-length tripartite SCs and duplicated but not-yet-separated SPBs (Fig. 4A). SCs may eventually begin to decay at late arrest points. SCs have been examined at a very late time point (15 h) in the *ndt80-1* mutant (Fig. 4B). The majority of nuclei still contained SCs, but the SCs were of variable thickness, suggesting some decay of lateral elements. In addition, virtually every nucleus contained a polycomplex. Polycomplexes comprise aggregated SC material and are very rare in isogenic wild-type cells (50, 51).

The meiotic defect of the *ndt80*Δ mutant is not dependent upon *SPO11* or *RAD50* function. In confirmation of the preliminary analysis of the *ndt80-1* mutant, the arrest conferred by *ndt80*Δ::*LEU2* is not dependent upon functions that act very early in the meiotic recombination pathway. While *spo11*Δ and *rad50*Δ mutants formed spores normally, *spo11*Δ *ndt80*Δ and *rad50*Δ *ndt80*Δ double mutants exhibited the same failure to undergo meiosis I and consequent defect in spore formation as observed in the *ndt80*Δ::*LEU2* single mutant (Table 3).

Reversibility of pachytene arrest. Pachytene-arrested *ndt80*Δ::*LEU2* cells are fully capable of returning to mitotic growth. Even after being held in pachytene in SPM for 24 h, 90% of the cells formed colonies when plated on growth medium (Fig. 3D).

The *ndt80*Δ mutant exhibits elevated levels of commitment to heteroallelic recombination. Commitment of cells to meiotic levels of recombination can be analyzed for cells that have not completed meiosis by plating meiotic cultures on a growth medium selective for recombinants of interest (17, 61). The *ndt80*Δ::*LEU2* mutation had essentially no effect on commitment to heteroallelic recombination at up to 5 h in SPM, at which time commitment to recombination reached maximal levels in a wild-type strain (Fig. 3D). Prolonged incubation of *ndt80*Δ::*LEU2* cells at pachytene arrest resulted in slightly elevated levels of commitment to gene conversion. In return-to-growth experiments, an *ndt80*Δ::*LEU2* diploid heteroallelic at *HIS4* (*his4X*/*his4B*) (NKY2310) reproducibly gave threefold more His^+ recombinants at 24 h than a wild-type strain (NKY2201): the frequency of gene conversion at *HIS4* was about 1.5% in a wild-type strain and 4.6% in the *ndt80*Δ mutant. The slight elevation in commitment to gene conversion in an *ndt80*Δ mutant is also observed at the *ARG4* locus: an *ndt80*Δ diploid heterozygous for closely linked *ARG4* markers, *arg4-Bgl* and *arg4-Nsp* (21), gave twice the level of $Arg4^+$

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                                                                    ctata  -361

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atgttacaatattatgtatacagagtaactagaagttctcttcggagatcttgaagttcacaaaagggaatcgat
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FIG. 2. *NDT80* sequence. The *NDT80* coding region and flanking sequences and the predicted amino acid sequence of the Ndt80 protein are shown. Also shown is the putative TATA transcriptional start signal.

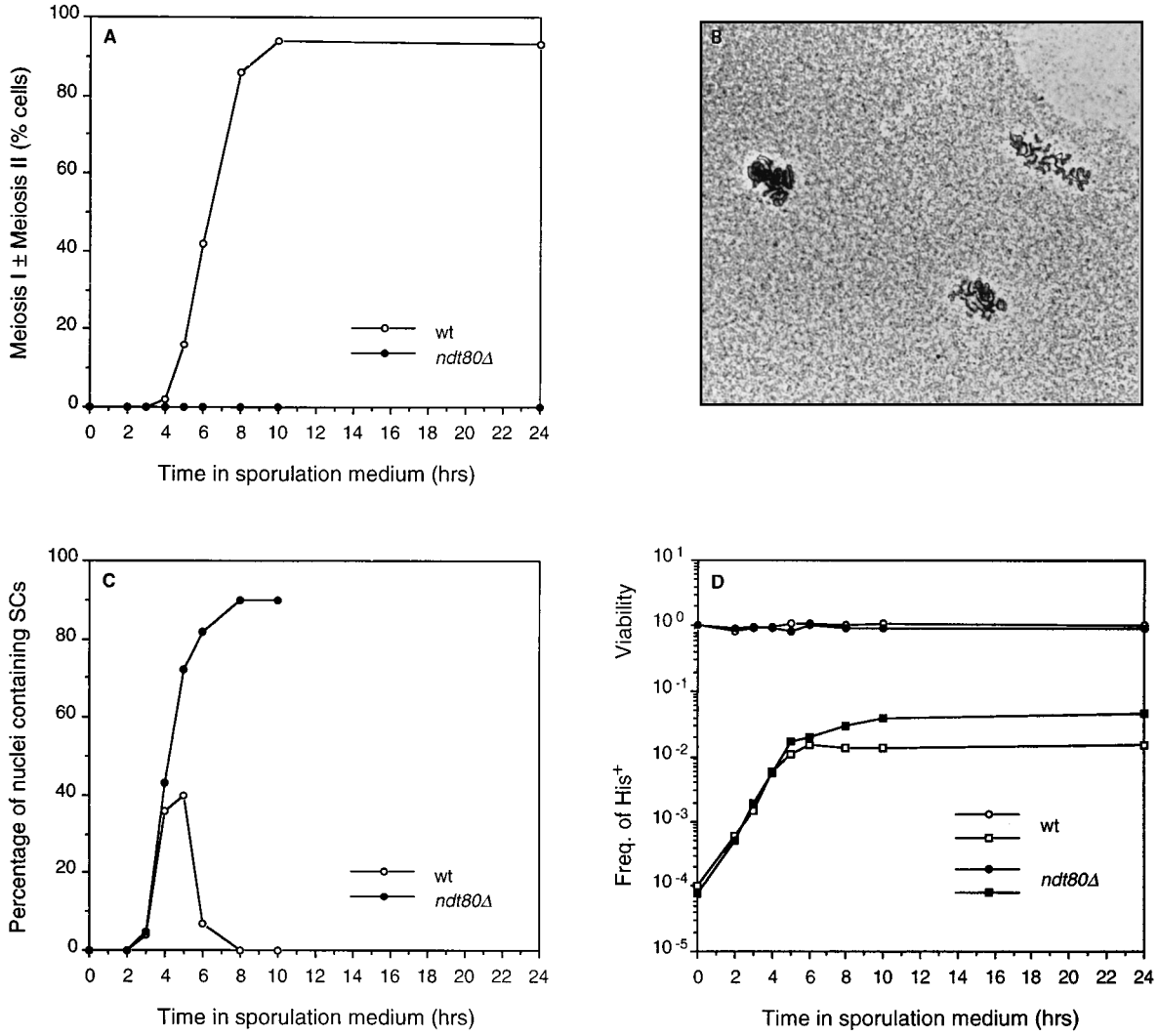

FIG. 3. Time course analysis of *ndt80*Δ mutant strain. Meiosis was initiated by transferring cells of strains NKY2201 (wild type [wt]) and NKY2310 (*ndt80*Δ) from YPA cultures into SPM. (A) The occurrence of meiotic divisions was monitored by DAPI staining and fluorescence microscopy. Cells that had completed a meiotic division(s) were bi- or tetranucleate and contained two (meiosis I) or four (meiosis I plus meiosis II) DAPI-staining bodies. Cells that failed to carry out meiosis remained mononucleate and contained only one DAPI-staining body. (B) The formation of SCs was examined by light microscopy. Shown are nuclei of an *ndt80*Δ strain containing SC structures at 5 h. (C) Percentages of nuclei containing SCs. In the wild-type strain, SCs were only transiently observed at 4 and 5 h. In the *ndt80*Δ cells, SCs were formed at the same time as in wild-type cells, but they accumulated: at 10 h, 90% of the *ndt80*Δ cells were at pachytene, whereas >90% of the wild-type cells had completed two meiotic divisions. (D) Meiotic cells were returned to vegetative growth by plating on synthetic complete medium. The viability was obtained by dividing the total number of CFU at the indicated times by the total number of CFU at time zero. Meiotic cells were also plated on histidine dropout medium to select His^+ recombinants. The frequency of recombination was determined by the ratio of His^+ CFU to total CFU at the indicated times after initiation of meiosis.

recombinants as did a wild-type strain (9.9% in NKY2647 versus 4.8% in NKY1464) (data not shown).

The *ndt80*Δ::*LEU2* mutant exhibits normal levels of DSB formation and 50% of the wild-type level of crossovers. An early step in many or all meiotic recombination events is the occurrence of site-specific DSBs, which have been observed at specific recombination hot spots and throughout the genome as a whole (12, 20, 70, 76, 82). These breaks occur transiently during prophase, prior to SC formation (51), and are then converted to interhomolog joint molecules which are now known to be double Holliday junctions (58, 59). Mature crossover and noncrossover products arise at approximately the end of pachytene (51, 68).

The effects of the *ndt80*Δ mutation on meiotic recombination have been examined at a recombination hot spot, the *HIS4LEU2* locus, where two meiosis-specific DSBs and high levels of reciprocal exchanges occur (12). Restriction fragments diagnostic of DSB formation were observed after digestion with *Pst*I and probing with *HIS4* distal sequence (probe 291; Fig. 5A). In the *ndt80*Δ mutant, normal levels of DSBs were formed in early prophase I, just as in wild-type cells (Fig. 5B). DSBs were not observed at 6 h in wild-type cells; however, residual levels of DSBs were still observable at a late time point (10 h) in an *ndt80*Δ::*LEU2* strain. The occurrence of crossovers is reflected in the appearance of two novel restriction fragments after *Xho*I digestion and probing with *HIS4* coding sequence (probe 155; Fig. 5A). Crossovers were also present in the *ndt80*Δ mutant, although at a somewhat reduced level: in a wild-type strain, recombinant fragments represented 20% of the total DNA, whereas in the *ndt80*Δ strain, recombinant bands constituted only 10% of the total DNA (Fig. 5C).

***NDT80* is a meiosis-specific gene.** Phenotypic analysis suggests that *NDT80* should function specifically in meiosis. Neither the *ndt80-1* nor the *ndt80*Δ::*LEU2* mutation has any dis-

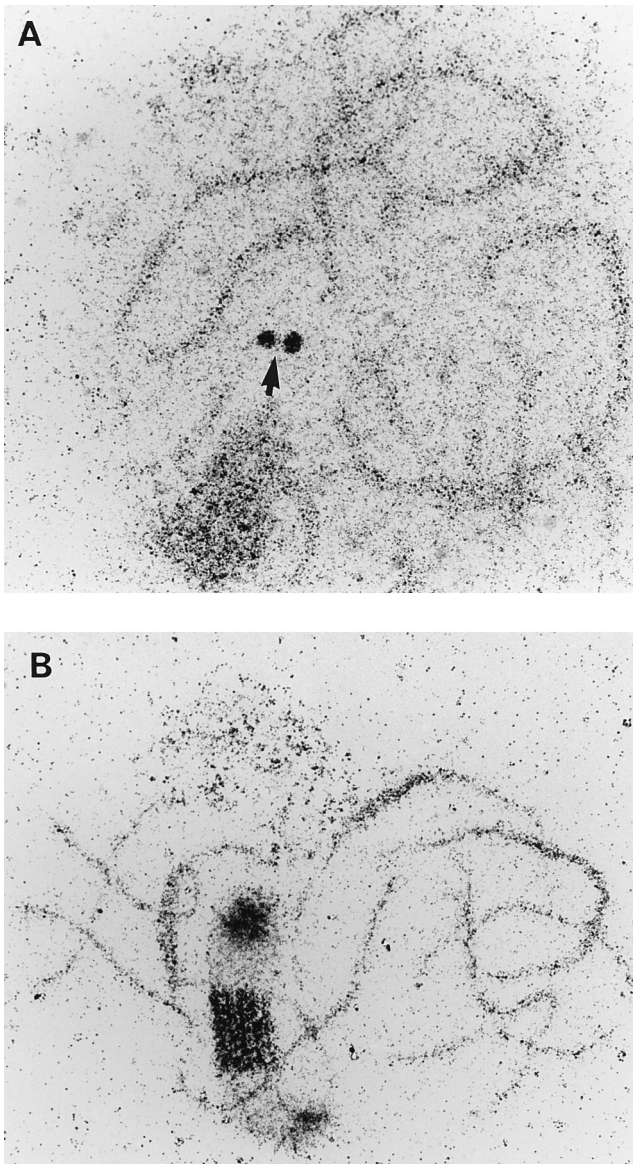

FIG. 4. Pachytene arrest of the *ndt80* mutants. The *ndt80*Δ::*LEU2* (NKY 2310) and the original *ndt80-1* (NKY2172) diploid strains were pregrown in YPA and then transferred to SPM. At 6 h (A) and 15 h (B) after initiation of meiosis, cells were collected and subjected to electron microscopy analysis according to the procedure of Loidl et al. (40). (A) Nucleus of the *ndt80*Δ::*LEU2* strain. All nuclei examined (26 of 26) exhibited full-length SCs and duplicated but unseparated SPBs (arrow). (B) Example of *ndt80-1* nuclei at a very late time point. Ninety percent of the nuclei of the *ndt80-1* strain contained SCs. At this point, wild-type cells had already completed two meiotic divisions and spore formation.

cernible effect on vegetative growth rate, colony size, or morphology. Furthermore, *ndt80* mutants are not sensitive to methylmethane sulfonate, a radiomimetic DNA-damaging agent (data not shown).

Northern blot analysis of *NDT80* RNA levels confirms the meiosis-specific nature of this gene (Fig. 6). No *NDT80* transcript could be detected in mitotic cells or in meiotic cells shortly after transfer to SPM. A dramatic increase in *NDT80* mRNA levels occurred in mid-prophase, at the time of leptotene and zygotene (3 to 4 h), with maximal accumulation at 6 h. The level of a control message, *ARG4*, remained essentially constant during this period (Fig. 6).

TABLE 3. *ndt80* arrest is not alleviated by a *spo11* or *rad50* mutation[a]

Strain	Relevant genotype	Cells completing one or both meiotic divisions (%) (10 h)	Sporulation (%) (24 h)
NKY611	*SPO11 RAD50 NDT80*	92	90
NKY2296	*SPO11 RAD50 ndt80*Δ	<0.5	<0.5
NKY2534	*spo11*Δ *RAD50 NDT80*	89	81
NKY2535	*spo11*Δ *RAD50 ndt80*Δ	<0.5	<0.5
NKY2536	*SPO11 rad50*Δ *NDT80*	87	65
NKY2537	*SPO11 rad50*Δ *ndt80*Δ	<0.5	<0.5

[a] Isogenic diploid strains homozygous for the indicated *ndt80*, *spo11*, and *rad50* mutations were analyzed. Meiosis was initiated by pregrowth in YPA medium followed by transfer into SPM. The occurrence of meiotic divisions was monitored by DAPI staining and fluoresence microscopy of samples taken at the indicated time after transfer to SPM. Cells that have completed one or both meiotic divisions contain two, three, or four DAPI-staining bodies. Spore formation was examined by phase-contrast microscopy. At least 200 cells were counted.

Meiosis-specific genes can be categorized as early, middle, or late according to the timing of their expression in meiosis (46). *NDT80* is probably a member of the middle class. The *NDT80* transcripts appear about 2 h later than those of an early meiotic gene, *DMC1* (Fig. 6).

DISCUSSION

Summary. *NDT80* is a new yeast gene which is required only during meiosis and is needed for exit from the pachytene stage. *NDT80* is transcribed in mid-prophase of meiosis and not in mitotic cells. *ndt80* cells arrested at pachytene remain viable and can return efficiently to vegetative growth. The *ndt80* arrest is not dependent upon *SPO11* or *RAD50* function. The *ndt80*Δ mutant is substantially normal for meiotic recombination but exhibits an enhanced commitment to heteroallelic recombination and a deficit in crossing over in real-time measurements.

Relationship of *ndt80* mutant phenotypes to those observed in other pachytene arrest mutants. A number of mutations that confer pachytene arrest have been examined with respect to the nature of meiotic recombination by genetic and/or physical assays.

The *ndt80* mutant phenotype is very similar to that conferred by mutations in the *CDC28* gene, even in relatively subtle aspects. *ndt80* and *cdc28* mutations both confer a modest elevation in the level of commitment to heteroallelic recombination (62; this work). Furthermore, in parallel examinations of isogenic SK1 strains, the *ndt80*Δ and *cdc28-63* mutations both confer a twofold reduction in the final level of crossovers (77a). Also, both are relatively normal with respect to the appearance and disappearance of meiosis-specific DSBs. Also, not unexpectedly, the *cdc28* mutant arrest is not dependent upon *SPO11* function (78).

An enhanced commitment to heteroallelic recombination and a deficit of crossovers have also been observed genetically in pachytene-arrested cells of strain AP-1 (15). It would be interesting to test whether AP-1 arrest is *SPO11* dependent.

The *ndt80* phenotype is also generally similar to that of an arrested *top2* mutant strain (53). In the latter case, commitment to gene conversion and recombination appear to occur at essentially normal levels, however.

The *ndt80*Δ phenotype is clearly distinct from those of most mutants that confer an intermediate block in recombination (see the introduction). *rad50S*, *dmc1*Δ, *sae1*, *sae2*, *sae3*, and *mei5* mutants all accumulate meiosis-specific DSBs (4, 9, 43,

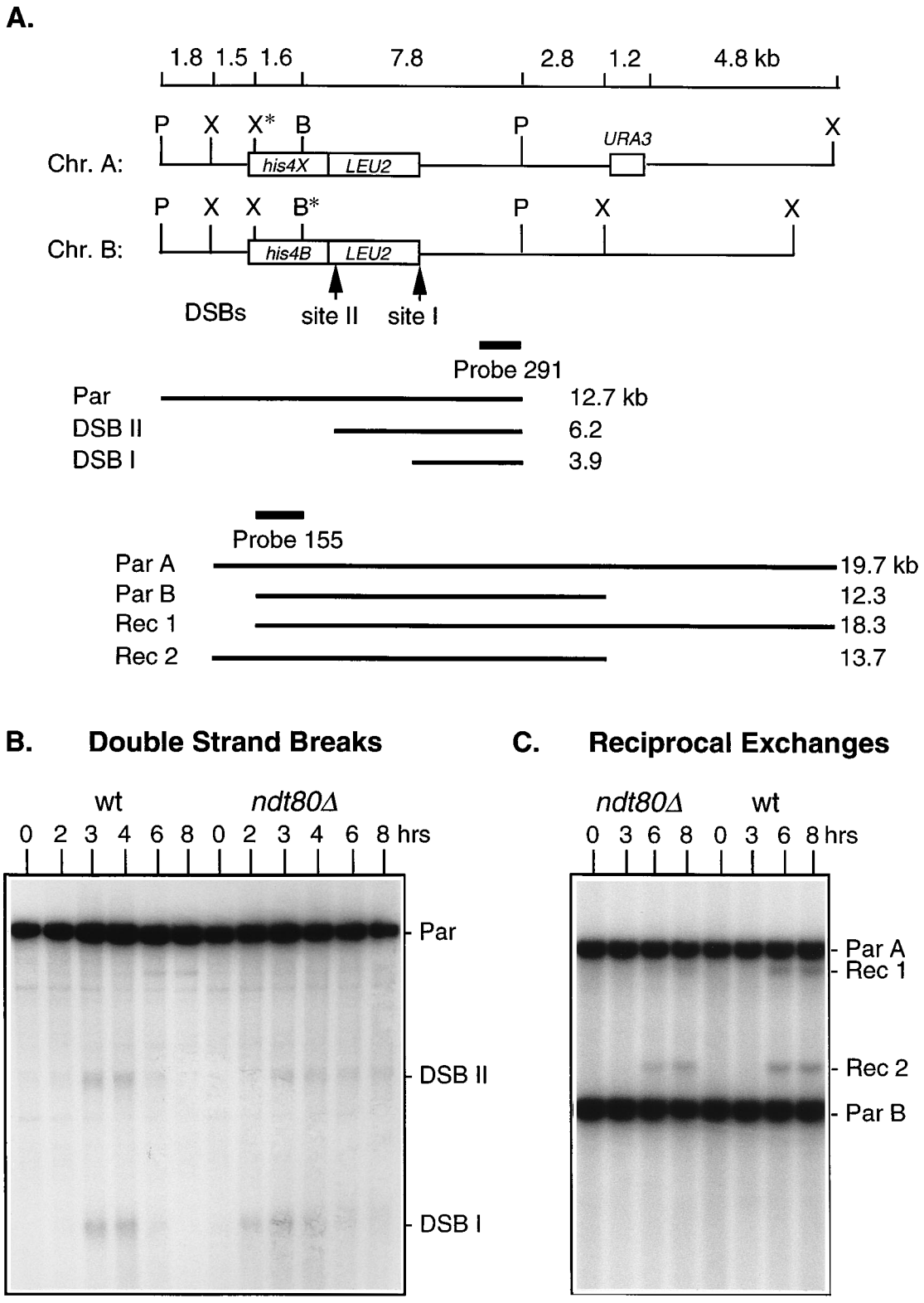

FIG. 5. Meiotic recombination in an *ndt80*Δ mutant. (A) Map of the *HIS4LEU2* construct. DNA fragments diagnostic of DSBs at site I (DSB I) and site II (DSB II) and the unbroken parental fragment (Par) are shown. Also shown are *Xho*I restriction fragments diagnostic of reciprocal exchanges (Rec 1 and Rec 2) and the nonrecombinant (parental) fragments (Par A and Par B). Chr., chromosome; P, *Pst*I; X, *Xho*I; B, *Bgl*II; ∗, restriction site destroyed. (B) DSB formation. DNA was isolated from meiotic cells of isogenic wild-type (wt) (NKY2201) and *ndt80*Δ (NKY2310) strains at the indicated times during meiosis. DNA was digested with *Pst*I, fractionated on a 0.8% agarose gel, transferred to a nylon membrane, and hybridized with probe 291. (C) Reciprocal recombination. The same DNA samples as in panel B were digested with *Xho*I, separated on a 0.6% agarose gel, and subjected to Southern analysis with probe 155.

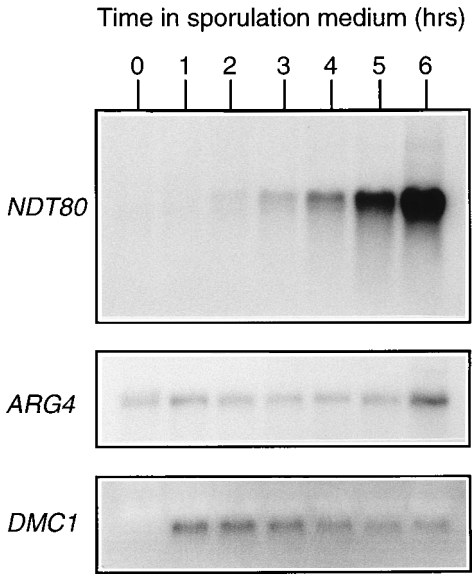

FIG. 6. Meiotic induction of the *NDT80* transcript. Total cellular RNA was isolated from cells taken at the indicated times after initiation of meiosis in a wild-type strain (NKY2201), fractionated on a 1.0% formaldehyde gel, and subjected to Northern blot analysis. The *NDT80* transcripts were detected by using the 1.2-kb *Eco*47III-*Bam*HI fragment of the *NDT80* gene (Fig. 1) as a probe. Transcription of the *ARG4* and *DMC1* genes, as controls, was also monitored during meiosis by using *ARG4* (2.0-kb *Sal*I-*Bgl*II fragment of pYe511) and *DMC1* (0.9-kb *Sty*I fragment of pNKY422) coding sequences as probes. While *ARG4* transcript levels remained constant during meiosis, transcription of *NDT80* and *DMC1* exhibited a dramatic induction during meiosis. It is noteworthy that the *NDT80* transcripts appear about 2 h later than those of the *DMC1* gene.

47). The *ndt80*Δ phenotype is also clearly distinct from that of an SK1 *zip1*Δ mutant, which is severely defective in the formation of crossovers at the time of normal meiotic prophase and yields crossover recombinants only much later (69).

Possible roles of *NDT80* during meiotic prophase. Unfortunately, sequence analysis has not provided any insight regarding the function of the *NDT80* gene in meiosis. Several possibilities can be considered. First, Ndt80 protein may be important in cell cycle regulatory processes; for example, it might influence the activity of MPF either directly or indirectly via effects on the expression of important components. The close similarity of the *ndt80* and *cdc28* phenotypes supports this possibility. The fact that the *NDT80* gene is expressed in the middle of prophase, along with other genes required during or after the transition out of pachytene, is also consistent with a role specifically at this point.

Second, Ndt80 might be a required component of some important aspect of meiotic prophase that is monitored by the pachytene arrest checkpoint. Since this aspect is not a gross disruption of meiotic recombination (see above), it might be either some feature of chromosome metabolism that has not yet been identified or some totally different process which is monitored by the pachytene checkpoint in addition to chromosome metabolism. We could speculate, for example, that cells monitor the accumulation of critical components required for completion of the two divisions and spore formation or monitor the presence of intermediate molecules required for synthesis of such components.

Third, if cells do use pachytene as a transition point when they return to mitotic growth in response to the presence of nutrients (see the introduction), the absence of Ndt80 might create a condition that mimics the presence of nutrients and thus causes pachytene arrest. Complete transition to vegetative growth might be blocked, however, because of the absence of additional required components.

Fourth, Ndt80 might be a structural component of meiotic chromosomes whose functioning is required for disassembly of SCs. This possibility is not highly likely, however. Neither a *spo11*Δ *ndt80*Δ nor a *rad50*Δ *ndt80*Δ double mutant makes SCs in the first place, but they still exhibit prophase arrest during meiosis. Moreover, since an *ndt80* mutant also fails to undergo SPB separation, this explanation requires in addition that SPB separation be dependent upon SC disassembly. However, separation of SPBs in cells containing full-length or partially disassembled SCs has been observed in some *cdc36* and *cdc5* mutant cells (57, 62, 64).

ACKNOWLEDGMENTS

We thank Andrew McKee for making available his collection of sporulation-defective mutants and members of the Kleckner laboratory for comments on the manuscript.

This work was supported by a grant (RO1-GM44794) to N.K. from the National Institutes of Health.

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