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The *dhp1*$^+$ gene, encoding a putative nuclear 5′→3′ exoribonuclease, is required for proper chromosome segregation in fission yeast

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ABSTRACT

The *Schizosaccharomyces pombe dhp1*$^+$ gene is an ortholog of the *Saccharomyces cerevisiae RAT1* gene, which encodes a nuclear 5′→3′ exoribonuclease, and is essential for cell viability. To clarify the cellular functions of the nuclear 5′→3′ exoribonuclease, we isolated and characterized a temperature-sensitive mutant of *dhp1* (*dhp1-1* mutant). The *dhp1-1* mutant showed nuclear accumulation of poly(A)$^+$ RNA at the restrictive temperature, as was already reported for the *rat1* mutant. Interestingly, the *dhp1-1* mutant exhibited aberrant chromosome segregation at the restrictive temperature. The *dhp1-1* cells frequently contained condensed chromosomes, most of whose sister chromatids failed to separate during mitosis despite normal mitotic spindle elongation. Finally, chromosomes were displaced or unequally segregated. As similar mitotic defects were also observed in Dhp1p-depleted cells, we concluded that *dhp1*$^+$ is required for proper chromosome segregation as well as for poly(A)$^+$ RNA metabolism in fission yeast. Furthermore, we isolated a multicopy suppressor of the *dhp1-1* mutant, referred to as *din1*$^+$. We found that the gene product of *dhp1-1* was unstable at high temperatures, but that reduced levels of Dhp1-1p could be suppressed by overexpressing Din1p at the restrictive temperature. Thus, Din1p may physically interact with Dhp1p and stabilize Dhp1p and/or restore its activity.

INTRODUCTION

In *Saccharomyces cerevisiae*, two 5′→3′ exoribonucleases have been isolated so far (1–3). One is encoded by the *RAT1* gene (4) [also referred as *HKE1* (1) and *TAP1* (5,6)] and the other is encoded by the *XRN1* gene (7) [also referred as *DST2* (8), *SEP1* (9), *KEM1* (10,11) and *RAR5* (12)]. They share homologous regions in their amino acid sequences and also similar biochemical properties such as exoribonuclease activity, but have different cellular functions. *RAT1* encodes a protein that is predominantly localized in the nucleus (1) and is essential for cell growth (1,4,6). In contrast, *XRN1* is not required for cell viability (7–10,12) and the gene product is localized to the cytoplasm (13). Xrn1p functions in turnover of mRNA (14–16) and pre-rRNA (17,18). Interestingly, when Xrn1p was localized in the nucleus by fusion of an NLS, it rescued the temperature-sensitivity of the *rat1-1* mutant. In contrast, wild-type Xrn1p did not, indicating that Rat1p and Xrn1p are functionally interchangeable proteins that normally reside and function in the nucleus and cytoplasm, respectively (19). Counterparts of Rat1p and Xrn1p have been identified in *Schizosaccharomyces pombe* (20,21), mouse (22–24) and human (25,26), indicating that these 5′→3′ exoribonucleases have been conserved throughout eukaryotes.

The *RAT1* gene was originally found to be involved in the export of poly(A)$^+$ RNA from the nucleus in *S.cerevisiae* (4). In the temperature-sensitive *rat1-1* mutant poly(A)$^+$ RNA rapidly accumulated in the nucleus after shift-up to the restrictive temperature (4). In addition, the *rat1-1* mutant displayed increased levels of a 5′-extended form of 5.8S rRNA, suggesting that the exoribonuclease activity of Rat1p is involved in the 5′-processing step of 5.8S rRNA maturation (4,17). Furthermore, Rat1p has been shown to be involved in the turnover and processing of nuclear RNA such as the degradation of several excised fragments of pre-rRNA spacer regions (27) and 5′-processing of small nucleolar RNAs (snoRNAs) (27–29), which are required for ribosome biosynthesis. However, the relationship between a defect in ribosome biosynthesis and the growth arrest observed for the *rat1* mutant remains unclear.

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Recent observations have identified a complex of 3′→5′ exoribonucleases, termed the exosome, which consists of at least 10 core subunits (Rrp4p, Rrp40p to Rrp46p, Mtr3p and Csl4p), a nuclear subunit (Rrp6p) and associated factors (Mtr4p, Ski2p, Ski3p and Ski8p) (30,31; reviewed in 32). All of the core subunits display sequence similarity with 3′→5′ exoribonucleases and some possess 3′→5′ exoribonuclease activity (30,31). Conditional defects in subunits of the exosome affect export of poly(A)$^+$ RNA from the nucleus (33) and 3′-processing of 5.8S rRNA (34–39), mRNA (40) and snoRNAs (35,41) at the restrictive temperature. The observations indicate that nuclear 3′→5′ (nuclear exosome) and 5′→3′ (Rat1p) exoribonuclease activities are involved in nucleocytoplasmic RNA trafficking and RNA processing (4,30,41). Besides their involvement in RNA metabolism, a mutation in the *S.pombe* ortholog of *RRP44*, termed *dis3-54*, was shown to result in defective chromosome segregation (42,43), suggesting involvement of nuclear exoribonucleases in chromosome segregation.

The *dhp1*$^+$ gene is an ortholog of *RAT1* in *S.pombe*. Both genes share 40% identity in their deduced amino acid sequences (20). Expression of Dhp1p rescued the temperature-sensitivity of a *rat1* mutant (20). In addition, overproduction of Dhp1p alleviated the defects of an *xrn1* mutant (slow growth and a reduced level of sporulation) as effectively as did Rat1p (20). These observations indicate that *dhp1*$^+$ is not only structurally but also functionally homologous to *RAT1*. The *dhp1*$^+$ gene disruptant spores germinate but fail to divide, showing that *dhp1*$^+$ is an essential gene (20). Although these results suggest that the *dhp1*$^+$ gene product is also an essential nuclear 5′→3′ exoribonuclease, the *in vivo* function of the Dhp1 protein has remained elusive, as is also the case for Rat1p of *S.cerevisiae*. In the present communication, we isolated and characterized a temperature-sensitive mutant of *dhp1*, in order to clarify the cellular functions of the *dhp1*$^+$ gene in fission yeast. We found that the *dhp1*$^+$ gene is required for proper chromosome segregation, as well as for poly(A)$^+$ RNA trafficking.

MATERIALS AND METHODS

Strains, media and plasmids

The *S.pombe* strains used in this study are listed in Table 1. Media and standard genetic procedures for *S.pombe* were performed as described by Alfa *et al*. (44). For transcriptional repression of *dhp1*$^+$ from pREP81-*dhp1*$^+$, thiamine was added to MM to yield a final concentration of 5 μg/ml. pREP81-*dhp1*$^+$, pREP41-*dhp1*$^+$ or pREP41-*din1*$^+$ was constructed by inserting the DNA fragment coding *dhp1*$^+$ or *din1*$^+$ downstream of the weak or medium *nmt1* promoter of pREP81 or pREP41 (45).

Sequential deletion analysis of Dhp1p

To generate C-terminal deletion mutants, the *Eco*47III–*Eco*RV (ΔC125) or *Eco*47III–*Acc*III (ΔC204) fragments of the *dhp1*$^+$ gene were inserted into the *Pst*I site of pDB248′ (46). For the N-terminal deletion mutant (ΔN91), the *Eco*47III–*Eco*T22I fragment lacking the *Pfl*MI–*Nco*I region was inserted into the *Pst*I site of pDB248′ as well. On each plasmid, the mutant gene is transcribed from the intrinsic promoter of *dhp1*$^+$. Procedures for expressing the deletion mutants in the *dhp1* null mutant using SSP1 were previously described (23).

Construction of the *dhp1-1* mutant

A 1.8 kb *ura4*$^+$ gene fragment was inserted into *Eco*RV site of pH8-2 (20). The resulting plasmid was subjected to restriction digestion by both *Nde*I and *Hin*dIII, and was then used for transformation of the *S.pombe* strain JY741 (*h*$^-$ *ade6-M210 leu1 ura4-D18*) to replace the chromosomal *dhp1*$^+$ gene with the truncated *dhp1* mutant gene via homologous recombination. Correct integration was confirmed by Southern blotting. The strain (MP102) was backcrossed once, and used in this study. MP102 was further crossed with MKY7A (*h*$^+$ *leu1 his7-lacI-GFP-his7*$^+$ *lys1-lacO-lys1*$^+$*ura4-D18*) to generate the KP38 strain used to visualize centromeric DNA–GFP (47).

Determination of growth rate and cell viability

The growth rate in mid-log phase cultures (10^6–10^7 cells/ml) at 36°C was monitored in duplicate by removing aliquots at intervals and counting the cell numbers with a haemocytometer. Simultaneously, properly diluted aliquots were plated onto YES plates and incubated for 4 days at 25°C to determine cell viability.

Fluorescence microscopy

Cells were stained with 4′,6-diamidino-2-phenylindole (DAPI) to visualize DNA in fixed cells and 3,3′-dihexyloxacarbocyanine iodide ($DiOC_6$) to visualize the nuclear envelope. Immunofluorescence microscopy using anti-tubulin antibody YOL1/34 (Sera-lab) and a rhodamine-conjugated goat anti-rat IgG (Immunotech S.A.) as secondary antibody was previously described (48). To visualize centromeric DNA–GFP, the cells (KP38) were fixed in 100% methanol at –70°C overnight, and then subjected to fluorescence microscopy after DAPI staining.

Table 1. *S.pombe* strains used

Strain	Genotype	Source
972	*h*$^-$	Our stock
MP102	*h*$^+$ *ade6-M216 leu1 ura4-D18 dhp1-1<<ura4*$^+$	This work
JY742	*h*$^+$ *ade6-M216 leu1 ura4-D18*	Our stock
SSP1	*h*$^+$/*h*$^-$ *ade6-M210/ade6-M216 leu1/leu1 ura4-D18/ura4-D18 dhp1*$^+$*/dhp1::ura4*$^+$	(20)
KP38	*h*$^+$ *ade6-M216 leu1 his7-lacI-GFP-his7*$^+$ *lys1-lacO-lys1*$^+$ *ura4-D18 dhp1-1<<ura4*$^+$	This work

Analysis of localization of poly(A)$^+$ RNA

Localization of poly(A)$^+$ RNA was analyzed by *in situ* fluorescence hybridization (FISH) as previously described (49).

Isolation of *din1*$^+$

The *S.pombe* genomic DNA library was introduced into the *dhp1-1* mutant by the lithium acetate method. The transformed cells were incubated at 25°C for 2 days, and then at 35°C for 5 days. Approximately 1.3×10^5 clones were screened and 11 clones that grew at 35°C were obtained. The plasmids were rescued in *Escherichia coli* and re-introduced into the *dhp1-1* cells to confirm their ability to suppress *dhp1-1* mutation. Nucleotide sequences of the inserts were determined by using an ABI PRISM 310 genetic analyzer. For the isolation of *din1*$^+$ cDNA, 5′- or 3′-rapid amplification of cDNA ends (RACE) with the *S.pombe* cDNA library was performed using the following primers: TGGGATCCAAAGGAGCGCAC for 5′-RACE and TCCTAAATCCGACCCTGATC for 3′-RACE. The nucleotide sequence of the *din1*$^+$ cDNA was determined as described above.

Western blotting

Rabbit anti-Dhp1p antiserum was obtained by standard procedures. Briefly, fusion proteins of maltose binding protein and full-length Dhp1p were expressed in *E.coli*. Whole cell lysates were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and the band of the over-produced protein was excised to immunize rabbits.

Fission yeast total soluble protein extracts were prepared using the glass bead method. Protein concentrations were determined by the Protein assay (Bio-Rad) using BSA as a standard. Equal amounts of protein were separated on each of the lanes of an SDS–polyacrylamide gel and transferred to polyvinylidene difluoride membranes, and western blotting was carried out with the Protoblot kit (Promega) with anti-Dhp1p (1:150 dilution) as the primary antibody.

RESULTS

Temperature-sensitivity of a mutant lacking the C-terminal segment of Dhp1p

The *dhp1*$^+$ gene is essential for cell viability (20). To determine the essential region of Dhp1p necessary for proliferation, truncated forms of the *dhp1*$^+$ gene were constructed by deleting carboxyl or amino portions of the gene on the *LEU2*-containing plasmid pDB248′. The plasmids were introduced into the heterozygous *dhp1*$^+$*/dhp1*::*ura4*$^+$ diploid strain SSP1, and the Ura$^+$ and Leu$^+$ haploid progeny, which contained both the chromosomal *dhp1* gene inactivated by the *ura4*$^+$ insertion and the truncated *dhp1* mutant gene on the plasmid, were selected for growth on minimal medium plates after sporulation. Dhp1ΔC125p, which had lost the C-terminal 125 amino acids of Dhp1p, rescued the viability of the *dhp1*::*ura4* cells, while neither Dhp1ΔC204p (lacking the C-terminal 204 amino acids) nor Dhp1ΔN91p (lacking the N-terminal 91 amino acids), could do so (Fig. 1). The results indicate that two highly conserved regions, HR1 and HR2, but not the 125 amino acid C-terminal portion, comprise the regions of Dhp1p necessary for cell growth. However, deletion of the C-terminal 125 amino acids of Dhp1p resulted in temperature-sensitive cell growth. When the chromosomal copy of *dhp1*$^+$ was truncated by the insertion of the *ura4*$^+$ gene, resulting in the loss of the C-terminal 125 amino acids (Fig. 2A), the mutant cell grew normally below 33°C, but not above 33°C (data not shown). We designated the mutation as *dhp1-1*. The *dhp1-1* mutant ceased to grow and lost viability 4 h after shift-up to 36°C in YES medium (Fig. 2B). In accordance with growth cessation, the mutant Dhp1-1p protein was quickly lost from the cells 3 h after shift-up (Fig. 2C), indicating that Dhp1-1p might be unstable at high temperatures. Introduction of a multicopy plasmid harboring the *dhp1-1* mutant gene suppressed the temperature-sensitivity of *dhp1-1* (data not shown). These data suggest that the temperature-sensitivity of the *dhp1-1* mutant was caused by a quantitative reduction in the amount of Dhp1-1p, possibly due to its instability at the restrictive temperature.

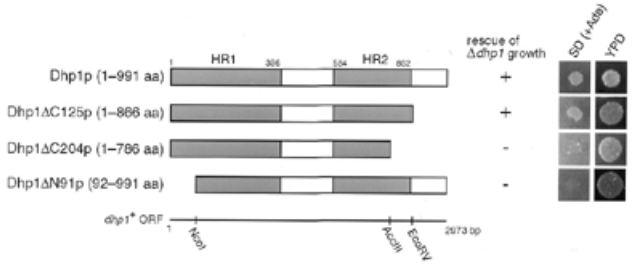

Figure 1. Sequential deletion analysis of Dhp1p. White bars, amino acid sequences of the truncated mutant Dhp1p; shaded bars [HR1 (1–396 amino acids) and HR2 (584–862 amino acids)], conserved regions between Dhp1p, Rat1p and mouse Dhm1p (23). Only relevant restriction sites in the *dhp1*$^+$ coding sequence are shown. Rescue of the Δ*dhp1* mutant by a series of truncated Dhp1 proteins was determined using spores from SSP1, which contain the *dhp1* alleles expressed from the *dhp1* promoter on the pDB248′ vector, depending on whether they can form colonies or not. Aliquots of spore suspensions, which carry plasmids expressing truncated forms of Dhp1p, were spotted on YPD plates and SD plates containing 10 μg/ml adenine [SD (+Ade)] and incubated at 30°C for 5 days. Spores with the *dhp1*$^+$ gene can form colonies only on YPD plates. Δ*dhp1* haploid spores (Ura$^+$ Ade$^-$) will form colonies on SD (+Ade) plates only when the *dhp1* deletion allele rescues cell growth. The data are presented to show both numbers of colonies that appeared (+) and failed to appear (−) on the plates.

Nuclear accumulation of poly(A)$^+$ RNA in the *dhp1-1* mutant

The *rat1-1* mutant was reported to accumulate poly(A)$^+$ RNA in the nucleus (4). To test whether the *dhp1*$^+$ gene is also involved in poly(A)$^+$ RNA metabolism in fission yeast, we performed FISH to analyze the localization of poly(A)$^+$ RNA in the *dhp1-1* mutant. The *dhp1-1* cells grown at 26°C were transferred to 37°C for 2 h, and then subjected to *in situ* hybridization with biotin-labeled oligo(dT)$_{50}$ and FITC–avidin. As shown in Figure 3, nuclear accumulation of poly(A)$^+$ RNA was observed in the *dhp1-1* cells at the restrictive temperature. In contrast, poly(A)$^+$ RNA was distributed throughout the wild-type cells under the same conditions. Thus, the *dhp1*$^+$ gene product is involved in poly(A)$^+$ RNA metabolism such as mRNA export from the nucleus or processing of poly(A)$^+$ RNA in the nucleus, as was also shown for Rat1p in budding yeast.

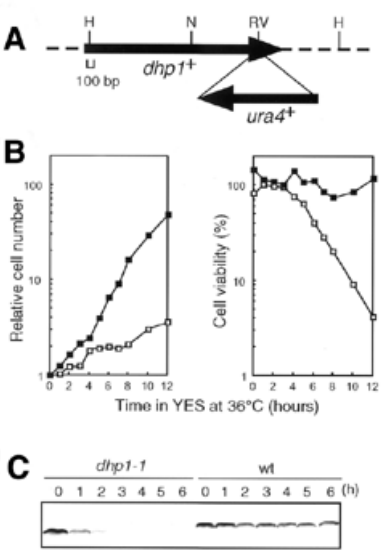

Figure 2. Properties of the *dhp1-1* mutation. (**A**) Construct of the *dhp1-1* allele. The *ura4*$^+$ gene was inserted into the *Eco*RV site of the *dhp1*$^+$ gene to generate Dhp1-1p, which lacks 125 amino acids from the C-terminus of Dhp1p. Only relevant restriction sites are shown. H, *Hin*dIII; N, *Nde*I; RV, *Eco*RV. (**B**) Growth rate of the *dhp1-1* mutant. Wild-type (JY742, closed squares) or *dhp1-1* (MP102, open squares) cells grown at 25°C in YES were cultured at 36°C. (**C**) Instability of Dhp1-1p at the restrictive temperature. Wild-type or the *dhp1-1* cells grown at 25°C in YES were cultured at 36°C. Soluble cell lysates prepared from aliquots were separated by 8.3% SDS–PAGE and subjected to western blotting with anti-Dhp1p antiserum.

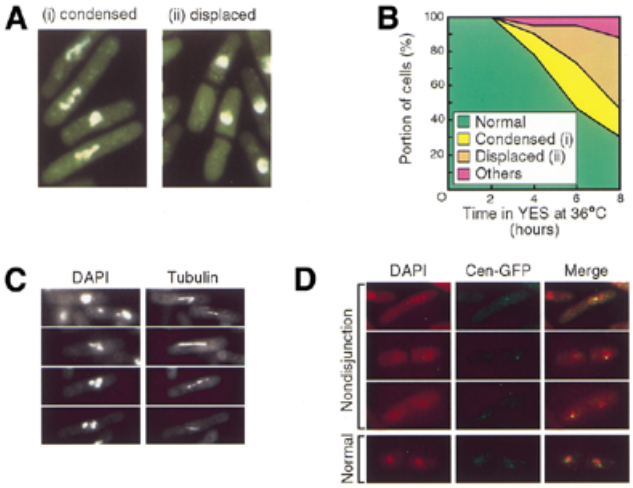

Figure 4. Defective chromosome segregation in the *dhp1-1* mutant. (**A**) Nuclear structures of the *dhp1-1* mutant at 36°C. *dhp1-1* (MP102) cells grown at 36°C for 6 h were stained with DAPI to visualize the nucleus. Typical nuclear morphologies of defective chromosome segregation are indicated: (i) cells with condensed chromosomes and (ii) cells with displaced nuclei. See also (C) and (D). (**B**) Frequency of the *dhp1-1* cells exhibiting defective chromosome segregation. The *dhp1-1* cells grown at 26°C were cultured at 36°C and aliquots were fixed with 70% ethanol and stained with DAPI. At least 120 cells were analyzed at every interval. (**C**) Elongating mitotic spindles that failed to segregate chromosomes. The *dhp1-1* cells cultured at 36°C for 6 h were subjected to immunofluorescence microscopy with the monoclonal anti-tubulin antibody YOL1/34. (**D**) Nondisjunction of sister chromatids in the *dhp1-1* mutant. *dhp1-1* cells (KP38) were cultured at 36°C for 6 h and the centromere DNA of chromosome I in the *dhp1-1* mutant was visualized by using the Cen1–GFP system.

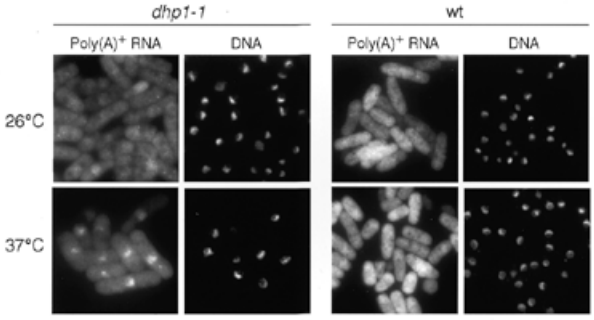

Figure 3. Analysis of poly(A)$^+$ RNA localization in the wild-type (wt) and *dhp1-1* cells. Wild-type (972) or *dhp1-1* (MP102) cells grown at 26°C were cultured at 37°C for 2 h. The cells were fixed and analyzed by *in situ* hybridization with a biotin-labeled oligo $(dT)_{50}$ probe. Hybridized signals were detected by FITC-conjugated avidin. Left panels show the poly(A)$^+$ RNA distribution, right panels (DNA) show the cells stained with DAPI.

Requirement of the *dhp1*$^+$ gene product for proper chromosome segregation

To characterize the temperature-sensitive *dhp1-1* mutant, the gross nuclear structure was analyzed in the *dhp1-1* cells at the restrictive temperature (Fig. 4). When the mutant was grown at 25°C, the majority of the cells exhibited interphase-like cell morphology, as observed for wild-type cells incubated at 25 or 36°C (data not shown). After a 3 h incubation at 36°C, however, cells exhibiting a variety of mitotic defects emerged in the *dhp1-1* mutant (Fig. 4B). This corresponded to the period during which cell viability also began to decrease (Fig. 2B). Some cells contained condensed chromosomes, which often formed the three blocks of nuclear material that are characteristic of the haploid karyotype of *S.pombe* (Fig. 4A). In other cells, the three blocks of condensed chromosomes appeared to be unequally pulled apart by the mitotic spindle (Fig. 4C) when separation of sister chromatids appeared to be hindered as reported for *dis* mutants (43). To investigate whether *dhp1-1* was deficient in sister chromatid separation, the Cen1–GFP system was used to visualize the centromere DNA of the chromosomes in the *dhp1-1* mutant (Fig. 4D). At the restrictive temperature, a single Cen1–GFP dot was frequently detected in only one of the three blocks of condensed chromosomes as well as in the unequally separated chromosomes (Fig. 4D, 'Nondisjunction'). Another fraction of the cells exhibits two Cen1–GFP signals in each nuclei, which seem to have executed normal separation of chromosomes (Fig. 4B and D, 'Normal'). On the contrary, the cells lacking disjunction of Cen1–GFP signals were not seen in the wild-type cells. These data suggest that disjunction of sister chromatids was impaired in the *dhp1-1* mutant. After prolonged incubation, the portion of cells with condensed chromosome decreased, while the fraction of the cells with a displaced nucleus increased (Fig. 4B). Anuclear cells were also observed. $DiOC_6$ staining of the cells revealed that the nuclear

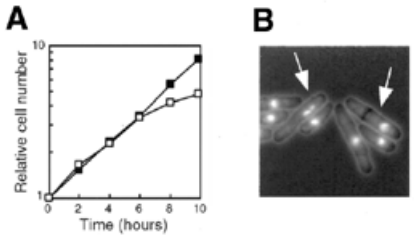

Figure 5. Depletion of Dhp1p from cells. (**A**) Growth of the Δ*dhp1* cells after shut-off of Dhp1p. Δ*dhp1* cells harboring pREP81-*dhp1*$^+$ were exponentially grown in thiamine-free MM (+Ade) and further incubated with (open squares) or without (closed squares) 5 μg/ml thiamine at 30°C. (**B**) Nuclear structure of Dhp1p-depleted cells. The Δ*dhp1* cells harboring pREP81-*dhp1*$^+$ were incubated for 10 h in the presence of thiamine and then fixed and stained with DAPI. Arrows indicate cells with defective chromosome segregation.

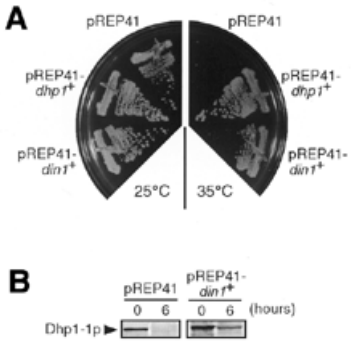

Figure 6. Suppression of the *dhp1-1* mutation by the *din1*$^+$ gene. (**A**) Abrogation of the temperature-sensitivity of *dhp1-1* by *din1*$^+$. The *dhp1-1* mutant (MP102) harboring pREP41, pREP41-*dhp1*$^+$ or pREP41-*din1*$^+$ was streaked onto MM (+Ade) plates and incubated at 25°C for 5 days or at 35°C for 2 days. (**B**) Rescue of the reduction in the Dhp1-1p level by Din1p overproduction at the restrictive temperature. The *dhp1-1* cells harboring pREP41 or pREP41-*din1*$^+$ were cultured in MM (+Ade) at 36°C. Soluble cell lysates prepared from aliquots were separated by 8.3% SDS–PAGE and subjected to western blotting with anti-Dhp1p antiserum.

envelope seemed to be normal in the *dhp1-1* cells at the restrictive temperature (data not shown). These results indicate that the *dhp1-1* mutant is defective in the normal execution of chromosome segregation. Taken together, it is suggested that the *dhp1-1* cells undergo a transient arrest in the cell number increase soon after shift-up (Fig. 2B, left), followed by cell division without chromosome segregation (Fig. 4A–D) and finally lose viability (Fig. 2B, right) in the period 3–4 h after shift-up.

Next, we investigated whether loss of Dhp1p also causes abnormal chromosome segregation in the *dhp1* null mutant. We constructed a *dhp1* null mutant harboring the pREP81-*dhp1*$^+$ plasmid, which carries the ORF for *dhp1*$^+$ under the control of the weak *nmt1* promoter Rep81, and grew the cells on thiamine-free plates. When Dhp1p expression was repressed by addition of thiamine, the cells ceased to grow (Fig. 5A) and exhibited chromosome segregation defects such as displacement of the nucleus (Fig. 5B), which was also observed in the *dhp1-1* mutant at 36°C. Thus, we concluded that the *dhp1*$^+$ gene is required for proper chromosome segregation.

Isolation of a multicopy suppressor gene for the *dhp1-1* mutation

To search for factors that interact with *dhp1*$^+$, we screened for multicopy suppressors of the *dhp1-1* mutation. The genomic library of *S.pombe* was introduced into the *dhp1-1* mutant and viable cells were selected at 35°C. Among the 1.3×10^5 clones screened, 11 clones were obtained. Nucleotide sequencing of the clones revealed that two genes were responsible for the suppression of temperature-sensitivity of the *dhp1-1* mutant. One of them, designated *din1*$^+$ (encoding the Dhp1p-interacting protein; DDBJ accession no. AB045607), was found to be identical to the uncharacterized ORF SPAC19D5.06c in the *S.pombe* chromosome I cosmid c19D5 (EMBL accession no. Z99531). The other is under investigation in our laboratory. A database search for homology revealed that Din1p had partial homology with the deduced amino acid sequence of *S.cerevisiae* YGL246c/*RAI1* (50), human *DOM3Z* (51) and *Caenorhabditis elegans dom-3* (52), suggesting that Din1p is conserved among eukaryotes.

When the plasmid containing the *din1*$^+$ gene (pREP41-*din1*$^+$) was introduced into the *dhp1-1* cells, the temperature-sensitivity was abrogated at 36°C, although the growth rate was slightly lower than that of wild-type cells growing at the same temperature (Fig. 6A). In addition, there was no significant loss of viability or any defective chromosome segregation in *dhp1-1* cells carrying pREP41-*din1*$^+$ at 36°C (data not shown). Furthermore, we found that the reduction in Dhp1-1p levels was considerably suppressed by pREP41-*din1*$^+$ at 36°C (Fig. 6B). Dhp1-1p was still present in *dhp1-1* cells carrying pREP41-*din1*$^+$ even after 6 h at 36°C, while it could not be detected under the same conditions in *dhp1-1* cells containing the vector alone. These data indicate that the *din1*$^+$ gene product can suppress the temperature-sensitivity of *dhp1-1* by preventing the loss of Dhp1-1p. During the preparation of this manuscript, Rai1p was reported to interact with, and stabilize the exoribonuclease activity of, Rat1p in *S.cerevisiae* (50), a result that is consistent with our observations in *S.pombe.*

To investigate the function of the *din1*$^+$ gene, we performed a gene disruption experiment of *din1*$^+$ and successfully obtained haploid cells lacking the *din1*$^+$ gene. The Δ*din1* mutant is viable even at the high temperature of 35°C (data not shown), indicating that the *din1*$^+$ gene is not essential for cell growth.

DISCUSSION

In the present study, we have characterized the fission yeast *dhp1*$^+$ gene, which encodes a putative exoribonuclease. Dhp1p has sequence similarity to Rat1p, and both of them are essential for cell viability (1,4,6,20). Since expression of *dhp1*$^+$ rescued the temperature-sensitivity of a *rat1* mutant, *dhp1*$^+$ is not only structurally, but also functionally homologous to *RAT1* (20). Rat1p is a nuclear protein that has 5′→3′ exoribonuclease activity (1). A series of analyses of *rat1* mutants revealed that Rat1p is involved in RNA trafficking and processing (4,17,27,28). The functional complementation of the *rat1* mutant by *dhp1*$^+$ expression indicates that Dhp1p is a putative

5′→3′ exoribonuclease. We previously identified a mouse ortholog of *dhp1*$^+$*/RAT1*, termed *Dhm1*, and showed that expression of the *Dhm1* cDNA in fission yeast could suppress the temperature-sensitivity of the *dhp1-1* allele as well as the lethality of the Δ*dhp1* allele (23). These data suggest that the functions of exoribonucleases of Rat1p/Dhp1p members are well conserved throughout eukaryotic evolution.

We found that Dhp1p, like Rat1p, is required for a certain type of RNA metabolism. Poly(A)$^+$ RNA accumulated in the nucleus of *dhp1-1* mutant at the restrictive temperature; this is also a characteristic of the budding yeast *rat1-1* mutant (4). This result is consistent with the idea that Dhp1p is a functional homolog of Rat1p and that it possesses 5′→3′ exoribonuclease activity and suggests that Dhp1p and Rat1p are required for the nuclear export of mRNA. However, another possibility is that the accumulated poly(A)$^+$ RNA corresponds to non-mRNA species that were aberrantly produced owing to defective 5′→3′ exoribonuclease activity. Recently, it was reported that aberrantly polyadenylated snoRNAs accumulate in budding yeast exosome mutants (41) that were originally isolated as mutants defective in mRNA export (33). The exosome was identified as a protein complex with 3′→5′ exoribonuclease activity required for RNA processing in budding yeast (30,31). The exoribonuclease activity of Rat1p is required for the processing of a variety of RNA species, suggesting that this may also be the case for Dhp1p. In support of the latter possibility, the cytoplasmic signal for poly(A)$^+$ RNA appeared not to be significantly reduced, irrespective of the level of nuclear accumulation of poly(A)$^+$ RNA in the *dhp1-1* mutant. Further research to identify the nature of the accumulated RNA species in the nucleus has to be done to address this issue in more detail.

Characterization of the *dhp1-1* mutant revealed involvement of the *dhp1*$^+$ gene in chromosome segregation. At the restrictive temperature, aberrant segregation of chromosomes was observed in the *dhp1-1* mutant but this has not been reported in *rat1* mutants. Some cells in the mutant cell population contained condensed chromosomes, which frequently consisted of three blocks of each chromosome that were pulled by the mitotic spindle. However, the sister chromatids appeared not to be fully separated, resulting in unequal or defective chromosome segregation. Analyses of chromosomal behavior using the Cen1–GFP system confirmed that *dhp1-1* was unable to separate sister chromatids efficiently. In addition, similar mitotic defects were observed in the *dhp1* null mutant, demonstrating that these phenotypes are not specific to the *dhp1-1* mutant. Thus, we conclude that *dhp1*$^+$ is required for the normal execution of chromosome segregation either directly or indirectly.

The chromosomal morphology of *dhp1-1* resembles that of *dis* mutants, which were isolated as being defective in the separation of the sister chromatids (43). They fell into at least three complementation groups, and the corresponding genes were determined. The *dis1*$^+$ gene encodes a microtubule-associating protein (53), while *dis2*$^+$ is a type I protein phosphatase (54,55). Interestingly, *dis3*$^+$ encodes a fission yeast ortholog of a component of the exosome (30,31,42). Although exoribonuclease activity of Dis3p or Dhp1p has not been experimentally determined, processing of some RNA species may be directly involved in the proper execution of chromosome segregation. However, it still remains unclear whether particular RNA species, and not just proteins, have a role to play in chromosome segregation. As mentioned above, it is possible that mRNA export might be defective in the *dhp1-1* mutant. Thus, defective chromosome segregation might be a secondary effect of defective mRNA export, which could lead to the depletion of proteins involved in the mitotic apparatus. In addition, it is also possible that Dhp1p may be directly involved in chromosome segregation by its distinct activity from that of exoribonuclease. Further study will be needed to elucidate the role of Dhp1p in chromosome segregation.

Finally, we isolated a novel gene, which genetically interacts with *dhp1*$^+$ in fission yeast. It was isolated as a multicopy suppressor of *dhp1-1* and named *din1*$^+$. Overproduction of Din1p rescued the temperature-sensitivity and defective chromosome segregation phenotype of *dhp1-1*, and prevented the reduction in the amount of the mutant Dhp1-1 protein. However, the lethality of the *dhp1* deletion mutant was not rescued by overproducing Din1p (data not shown). These results indicate that Din1p overexpression rescued the temperature-sensitivity of *dhp1-1* by maintaining nearly normal levels of mutant Dhp1-1p in the cells. Din1p is likely to interact with, and stabilize, Dhp1p although one cannot rule out the possibility that overproduction of Din1p affects the level of *dhp1-1* mRNA or efficiency of Dhp1-1p translation. The C-terminal domain of Dhp1p may be required for efficient interaction with Din1p. The idea that Din1p physically interacts with Dhp1p is supported by very recent work in *S.cerevisiae*. Rai1p, an ortholog of Din1p, was found to bind to Rat1p, an ortholog of Dhp1p (50). Gene disruption of *din1*$^+$ revealed that, unlike *dhp1*$^+$, it is not essential for cell viability, indicating that *din1*$^+$ is dispensable for the essential function of *dhp1*$^+$. The *rai1* null mutant is also viable in *S.cerevisiae*, although the null mutation allele of *rai1* confers slow growth on the cells. These data suggest that Din1p may be an auxiliary protein that stabilizes Dhp1p and/or facilitates its activity. Furthermore, the roles of Din1p may be compensated by other proteins that are functionally redundant. It is possible that Dhp1p may form an exosome-like complex with Din1p and other proteins. This idea would gain further credence if other factors that interact with *dhp1*$^+$ were identified.

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