Ded1 is a fission yeast DEAD box protein involved in translation. We isolated Ded1 in a screen for multi-copy suppressors of a cold-sensitive, loss-of-function mutant of the cyclin-dependent kinase Cdc2. The checkpoint protein kinase Chk1, required for cell cycle arrest in response to DNA damage, was also isolated in this screen. Ded1 interacts with Chk1 in a two-hybrid screen, and this physical interaction can be recapitulated in Schizosaccharomyces pombe. The Ded1 polypeptide is modified in response to heat shock and depletion of carbon source. These two stressors appear to cause different modifications. Thus, the Ded1 protein appears to respond to particular types of cellular stress and may influence the activity of Cdc2 as a result.

Cdc2, a universally conserved protein kinase, is the primary cyclin-dependent kinase that regulates progression through the cell cycle in the fission yeast, Schizosaccharomyces pombe (1). Cdc2 is subject to regulation at multiple levels, including association with a cyclin subunit and phosphorylation at several sites that confer activation or inhibition (2). Association with a B-type cyclin such as the one encoded by fission yeast cdc13 is critical for Cdc2 activity, as is phosphorylation on a threonine residue (Thr167). Cdc2 is maintained in an inactive state by phosphorylation of a tyrosine residue at position 15 by the kinases Wee1 and Mih1. Activation of Cdc2 at the time of mitotic entry is achieved via dephosphorylation of tyrosine 15 by the protein-tyrosine phosphatase, Cdc25.

Elimination of tyrosine phosphorylation of Cdc2 causes cells to enter a catastrophic mitosis (3). This can be achieved in fission yeast by creating a strain in which the nonessential mik1 gene is disrupted and a temperature-sensitive allele of wee1 is inactivated by incubating cells at the restrictive temperature (Ref. 4; see Fig. 1A). Even cells that are blocked in S phase because of depletion of nucleotide pools by treatment with the ribonucleotide reductase inhibitor hydroxyurea will enter mitosis if Mik1 and Wee1 function are simultaneously lost. The phenotype of mitotic catastrophe caused by loss of Mik1 and Wee1 function can be suppressed by a conditional, cold-sensitive, loss-of-function allele of cdc2, known as cdc2-r4 (4).

In an effort to further our understanding of Cdc2 regulation in S. pombe, a multi-copy plasmid library was introduced into the cdc2-r4 strain to identify multi-copy suppressors of the cold-sensitive phenotype. The protein kinase Chk1, which plays a critical role in the checkpoint response to DNA damage, was identified in this screen (5). In addition, a plasmid encompassing a gene, ded1, with homology to the DEAD box family of RNA helicases conferred suppression. The amino acid sequence aspartic acid, glutamic acid, alanine, and aspartic acid (dEAD) is characteristic of this family of proteins. Members of this family have been proposed to carry out functions ranging from splicing to chromatin remodeling to translational initiation (6). The most closely related homologue of ded1 in the budding yeast is DED1, which has been shown to be important in initiation of translation (7, 8). Recently, Saccharomyces cerevisiae Ded1 has been shown to possess RNA helicase activity (9).

In both yeasts, ded1/DED1 is essential (data not shown and Ref. 10).

The S. pombe ded1 gene subsequently emerged from a number of genetic screens (11–13). Forbes et al. (12) identified ded1 (sum3) in a screen for dosage suppressors of the lethality caused by overexpression of Cdc25 in the presence of the ribonucleotide reductase inhibitor hydroxyurea. Kawamukai (13) identified ded1 (noc2) in a screen for dosage suppressors of sterile S. pombe mutants. The ded1 gene suppressed a strain in which sterility resulted from elevated levels of cAMP, as well as a strain in which sterility resulted from deregulated Ras function. Finally, Grallert et al. (11) identified mutant alleles of ded1 in two different genetic screens. In one, cold-sensitive ded1 alleles suppressed the temperature-sensitive loss of function of cdc19, an MCM protein family member. In a separate screen, temperature-sensitive alleles of ded1 were identified that caused cell cycle arrest. Interestingly, inactivation of ded1 causes arrest of cells at two points in the cell cycle, with either a 1 or a 2 N DNA content, reminiscent of the phenotype of cdc2 mutants.

In a two-hybrid screen to search for proteins that interact with Chk1 (14), we identified the gene encoding Ded1. Thus, two proteins that independently confer survival on a mutant lacking Cdc2 function are able to physically interact with one another. High copy suppression of the cdc2-r4 mutant uncovered a checkpoint response protein as well as a translation factor. The DNA damage checkpoint pathway clearly impinges on cell cycle control through the regulation of Cdc2. Ded1 is an essential gene, the loss of function of which leads to cell cycle arrest in a manner that is reminiscent of cells lacking Cdc2 function (11). We show here that the Ded1 polypeptide is responsive to stress conditions that affect translation and that could impact on cell cycle progression. Interestingly, the modified form of Ded1 that is generated in response to heat shock stress is still found in cells with mutations in the mitogen-activated protein kinase stress response signal transduction pathway, suggesting that the Ded1 response is independent of that pathway or operates upstream of it.
Ded1 Interacts with Chk1 and Cdc2

TABLE I
Strains used in this study

| Strain | Genotype | Source |
|--------|----------|--------|
| 972    | h        | Lab stock |
| SP630  | h,cdc25-22 leu1-32 | Lab stock |
| NW80   | h,cdc2-r4 leu1-32 | Lab stock |
| NW111  | h,chk1::ura4 ura4-D18 | Lab stock |
| NW158  | h,chk1::ura4 ura4-D18 leu1-2 ade6-216 | Lab stock |
| NW175  | h,cdc2-r4 chk1∷ura4 ura4-D18 leu1-32 | Lab stock |
| NW345  | h,sty1∷ura4 leu1-32 ura4-D18 ade6-210 | Lab stock |
| NW346  | h,vis1∷ura4 leu1-32 ura4-D18 | J. Millar |
| NW347  | h, wak1∷ura4 leu1-32 ura4-D18 ade6-216 his7-366 | J. Millar |
| NW348  | h, wak1∷LEU2 leu1-32 ura4-D18 ade6-M210 his7-366 | J. Millar |
| NW349  | h, mec4∷his7 leu1-32 ura4-D18 ade6-M210 his7-366 | J. Millar |
| NW350  | h, win1-1 leu1∷ura4 ura4-D18 ade6-M210 his7-366 | J. Millar |
| NW351  | h, wak1∷ura4 win1-1 leu1-32 ura4-D18 ade6-M210 his7-366 | J. Millar |
| NW352  | h, mec4∷ura4 win1-1 leu1-32 ura4-D18 ade6-M210 his7-366 | J. Millar |
| NW353  | h, atf1∷ura4 leu1-32 ura4-D18 his7-366 | J. Millar |
| NW354  | h, pap1∷ura4 leu1-32 ura4-D18 | J. Millar |

EXPERIMENTAL PROCEDURES

Yeast Strains, Media, and Plasmids—The S. pombe strains used in this study are listed in Table I. The cells were grown in YEA medium (15) at 30 °C except as described. Standard genetic methods were utilized, and most strains were constructed by random spore analysis.

The genomic ded1 gene was first cloning from a pWIIU-based genomic library in a screen of multicity suppressors of cdc2-r4 described elsewhere (5). A 3-kb HindIII/SalI fragment was cloned into the pSP1 vector (16) at the HindIII and PstI sites and was shown to suppress the cold sensitivity of cdc2-r4. Genetic alleles of cdc2 and cdc13 used in cdc2-r4 rescue experiments were also cloned in pSP1. The full-length chk1 plasmid and protocol for the two-hybrid screen were described previously (14). A plasmid containing an N-terminally truncated allele of the ded1 cDNA was identified from the two-hybrid screen with Chk1 (14). The resulting protein lacks the first 170 amino acids of Ded1. The full-length ded1 cDNA was isolated from an S. pombe cDNA library and amplified by PCR for cloning into pACT. For overexpression of ded1, the cDNA was cloned into the pREP81 vector containing the thiamine-repressible nmt1 promoter at the NdeI and SalI sites. To induce expression, yeast bearing the plasmid were grown on minimal medium containing thiamine to mid-log phase, washed three times in thiamine-free medium, and then resuspended in medium without thiamine for 18–22 h. For the cell cycle synchronization experiment, a temperature-sensitive cdc25-22 strain was used. Mid-log phase cells were arrested for 4 h at 36 °C and then shifted to 25 °C. Aliquots were taken every 20 min for the preparation of lysates and for fixation and staining with Calcofluor (Sigma-Aldrich) to determine the septation index.

Preparation of Antibody to Ded1—The full-length ded1 cDNA was cloned in-frame into the pGEX-3X vector at the HI site. The resulting glutathione S-transferase fusion protein was purified on and eluted from glutathione-Sepharose, as described by the manufacturer (Amersham Biosciences, Inc.). Soluble protein was used to generate rabbit polyclonal antibody (Cocalico Biologics).

Preparation of Lysates and Immunoblotting—For Western blot analysis of total cell extracts, the cells were harvested by centrifugation and lysed in phosphate-buffered saline (17) using glass beads. The cells were broken using the FastPrep machine (Bio101, Savant) at a setting of 6.5 for two bursts of 15 s. Following a 3,000 rpm spin in a microcentrifuge for 2 min, the supernatant was collected for protein determination using the Bradford assay method (Bio-Rad). Aliquots were run on 8% SDS-PAGE, transferred to nitrocellulose (Schleicher & Schuell, BA83), and probed with antibody. Blocking of the membranes and all antibody incubations and washes were done with 1% milk and 0.05% Tween 20 in phosphate-buffered saline. Peroxidase-coupled secondary antibodies (Roche Molecular Biochemicals) and the enhanced chemiluminescence detection system from PerkinElmer Life Sciences were used to detect the immune complexes. The polyclonal Ded1 antibody was used at a dilution of 1:50,000. HA-tagged Chk1 (18) was detected with 12CA5 antibody to the HA epitope at a 1:1,000 dilution. Cdc2 was detected with anti-PSTAIRE antibody (Santa Cruz). For immunoblotting of Cdc13 protein, lysates were prepared in 10 mM tris and boiled in water for 5 min before lysing with glass beads as described above. The antibody to Cdc13 (SP4) was generously provided by Sergio Moreno (University of Salamanca).

FIG. 1 . Isolation of ded1 as a multicopy suppressor of cdc2-r4.
A. phenotypes of strains relevant to the screen for multicopy suppressors of cdc2-r4 (pSec1). Simultaneous inactivation of nmt1 and wee1 results in mitotic catastrophe that can be suppressed by a mutation in cdc2, cdc2-r4 (4). The cdc2-r4 allele is itself cold-sensitive. Plasmids that suppress loss-of-function of cdc2-r4 were isolated and include plasmids that encode Ded1 and Chk1 (5).
B. a fixed number of cells of the cdc2-r4 and cdc2-r4 chk1∷ura4 strains transformed with the indicated plasmids were plated, incubated at 20 °C for the period of time indicated, and then shifted back to 30 °C. Survival was determined after 3 days by comparing the colony number relative to plates incubated continuously at 30 °C. Increased expression of Ded1 does not perceptibly alter the protein level of Cdc13. The cdc2-r4 strains transformed with the indicated plasmids were grown to mid-log phase, lysed in urea, and analyzed by Western blot with antibody to Cdc13 (SP4).

Stress Treatments—For heat shock treatment, the cells were grown to mid-log phase at 30 °C and then transferred to a preheated 42 °C water bath for the time indicated. The cells were either collected by centrifugation or returned to 30 °C for the time indicated before harvesting. To test the response to osmotic stress, mid-log phase cells were washed once in rich medium containing either 5 M KCl or 1 M sorbitol, resuspended in the same medium, and incubated for the time indicated. For nutrient starvation cells were grown to mid-log phase in minimal medium. Cells harvested by centrifugation were washed twice and then resuspended in minimal medium lacking either nitrogen source or carbon source (15). The cultures were incubated for the time indicated and harvested by centrifugation, and lysates were made as described above. For exposure to DNA damage generated by camptothecin (CPT), cells were grown to mid-log phase, then 40 μM CPT was added, and...
incubation was continued for 3 h. Camptothecin lactone was obtained from the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health. CPT was dissolved in Me2SO to 10 mM, and aliquots were stored at −10°C. For exposure to UV light, mid-log phase cells were plated on rich medium and exposed to 100 J/m2 of UV light, collected from the plate, and then incubated for 30 min in liquid culture before harvesting. To block DNA replication, mid-log phase cells were exposed to 12 mM hydroxyurea for 2 h and then harvested. To generate oxidative stress, mid-log phase cells were incubated in 5 mM H2O2 for 30 min and then harvested. To block protein synthesis, the cells were incubated in the presence of 100 μg/ml cycloheximide (Sigma-Aldrich) for 30, 60, or 120 min prior to heat shock treatment.

RESULTS

Identification of Ded1 as a Multi-copy Suppressor of cdc2-r4—Suppression of mitotic catastrophe conferred by the loss of Mik1 and Wee1 function can be achieved with a mutation in cdc2 (Ref. 4 and Fig. 1A). The cdc2-r4 allele was cloned by PCR and sequenced. The only mutation in cdc2-r4 is a Gt to A nucleotide change resulting in an amino acid change from aspartic acid to asparagine at position 90. Interestingly, the same cold-sensitive mutant allele of cdc2 was uncovered in an independent genetic screen for suppressors of mitotic catastrophe caused by the overexpression of cdc25 in combination with a temperature-sensitive wee1-50 allele (19). The histone H1 kinase activity of the Cdc2D90N protein is reduced relative to wild-type Cdc2 protein when assayed at a permissive (29 °C) or nonpermissive (20 °C) temperature (19).

To identify additional regulators of the Cdc2 cell cycle control pathway, we looked for dosage suppressors of the cold-sensitive cdc2-r4 allele (Fig. 1A and "Experimental Procedures"). Plasmids that allowed the cdc2-r4 strain to form colonies at 18 °C were recovered and sequenced. One plasmid encoded the DNA damage checkpoint kinase Chk1 (5). Another gene, originally dubbed soc1 (for suppressor of cdc2) but now called ded1, encodes a member of the DEAD box protein family. In addition, a plasmid containing the cdc13 gene confers very good suppression as measured by colony formation (data not shown).

To test whether the ability of ded1 to rescue the cold-sensitive phenotype of cdc2-r4 was acting through the chk1 pathway, plasmids were introduced into a cdc2-r4 chk1::ura4 strain and tested for growth at 20 °C. The ability of ded1 to support colony formation in this strain background was variable (data not shown). Therefore, we used an alternative method to evaluate suppression. The cdc2-r4 and cdc2-r4 chk1::ura4 strains were transformed with plasmids containing genomic alleles of cdc2, cdc13, ded1, or chk1 or vector alone. Transformants were grown in liquid culture, and ali-
quots were plated. The plates were incubated at 20 °C for various periods of time and then transferred to the permissive temperature of 30 °C to allow colony formation. This assay measures the ability of the plasmids to allow the strains to survive exposure to restrictive temperature. As shown in Fig. 1B, Ded1, Chk1, and Cdc13 all rescue with about the same efficiency. Furthermore, Ded1 and Cdc13 rescue of cdc2-r4 does not require Chk1 function (Fig. 1B).

The B-type cyclin Cdc13 is periodically synthesized and destroyed during the cell cycle. *S. pombe* Ded1 is a putative RNA helicase that may play an important role in protein translation. We therefore considered the possibility that increased expression of Ded1 rescued cdc2-r4 indirectly by increasing the level of Cdc13 protein. Indeed, Grallert et al. (11) demonstrated that loss of Ded1 function in *S. pombe* results in a preferential reduction in the amount of Cdc13 as compared with bulk proteins. Levels of Cdc13 are also disproportionately sensitive to inactivation of another DEAD box helicase, the translation initiation factor 4A (eIF4A) homologue of fission yeast, Tif1 (20). To test the possibility that Ded1 might rescue cdc2-r4 by increasing the amount of Cdc13 protein in the cell, we made lysates of cdc2-r4 strains transformed with plasmids to express Ded1, Cdc13, Cdc2, and Chk1 and measured the amount of Cdc13 in the cells by immunoblot. As shown in Fig. 1C, neither expression of Chk1 nor Ded1 affects Cdc13 levels.

**Identification of Ded1 as a Chk1-interacting Protein**—The fact that Ded1 and Chk1 both rescue the cold-sensitive phenotype of cdc2-r4 might indicate that they participate on a common pathway. In a search for proteins that interact with Chk1, we identified a clone that expresses a truncated allele of Ded1 that is missing the first 170 amino acids. Full-length Ded1 fused to the Gal4 activation domain interacts with a Chk1-Gal4 DNA-binding domain fusion as well (Fig. 2A). Using a series of truncated versions of Chk1 that separate the conserved kinase domain from the C-terminal noncatalytic domain, we determined that Ded1 interacts with the noncatalytic domain of Chk1. Furthermore, the N-terminal, nonhelicase domain of Ded1 is not required for this interaction.

To confirm that Chk1 and Ded1 can interact *in vivo*, co-immunoprecipitation experiments were performed. When Ded1
and HA-tagged Chk1 were expressed in single copy from the genome, no reproducible interaction could be detected. Failure to observe an interaction may be the result of the low abundance of Chk1 in fission yeast. Therefore, we employed a multiplicity vector (pSP1 (16)) to increase expression of HA-tagged Chk1 – 20-fold (data not shown). When Chk1 was immunoprecipitated from these cells with antibody to the HA epitope, Ded1 was found to co-precipitate by immunoblot (Fig. 2B). Thus, Ded1 and Chk1 are capable of interacting in fission yeast cells.

Previous studies have demonstrated that Chk1 is phosphorylated following DNA damage (18, 21). As shown in Fig. 2B, two forms of Chk1 are present in cells treated with the topoisomerase I poison CPT. Chk1 association with the 14-3-3 protein Rad24 is stimulated by DNA damage (14). In contrast, the association of Ded1 with Chk1 was not significantly affected by DNA damage (Fig. 2B). Furthermore, no change in mobility or abundance of Ded1 is apparent after DNA damage.

Standard genetic procedures were used to generate a disrupted allele of ded1 in a diploid. Tetrad analysis indicates that ded1 is an essential gene (data not shown and Ref. 13). Overexpression of ded1 causes cell elongation (Ref. 12 and data not shown) similar to the effect of overexpression of chk1 (5). However, cells tolerate overexpression of ded1, whereas overexpression of chk1 is lethal. To test whether chk1 function is required for ded1 to cause cell elongation, ded1 was overexpressed in a chk1 disruption strain. No visible difference between wild-type and chk1::ura4 cells was observed when ded1 was overexpressed (data not shown).

To determine whether the abundance or mobility of Ded1 varies during the cell cycle, a synchronous culture of cells was prepared (“Experimental Procedures”). Aliquots of cells were examined for septation index as an indicator of passage through the cell cycle. Lysates were prepared at each time point and probed with antibody to Ded1 and to Cdc2, a protein whose abundance does not vary during the cell cycle. As shown in Fig. 3, Ded1 abundance and mobility are stable, with no dramatic variation during the cell cycle.

**An Alternative Mobility Form of Ded1 Is Apparent Following Heat Shock and Is Independent of the Mitogen-activated Protein Kinase Pathway**—Studies from other investigators suggest that Ded1 plays an important role in the initiation of translation (8). Translation is regulated in response to various stress conditions. For example, transient exposure to high temperature (42 °C, heat shock) inhibits translation in eukaryotic cells (22). To determine whether Ded1 might be responsive to stress conditions that affect translation, we examined the behavior of the Ded1 protein following heat shock treatment. As shown in Fig. 4, an alternative form of Ded1 is apparent within 30 min of exposure to high temperature that is revealed as an alteration of mobility on SDS-PAGE (Fig. 4A). The alternative form of Ded1 gradually disappears when cells are shifted back to a normal growth temperature of 30 °C (Fig. 4B). The appearance and disappearance of the alternative form of Ded1 are not dependent on the presence of Chk1 (Fig. 4, A and B). To determine whether Chk1 is required for cell survival upon exposure to heat shock, a strain with the chk1 gene deleted was compared with wild-type cells and a heat shock-sensitive strain, sty1::ura4, for colony forming ability following transient exposure to high temperature. As shown in Fig. 4C, the chk1::ura4 strain behaves like the wild-type strain, suggesting that Chk1 is not required for survival following heat shock.

A number of proteins have been identified that are involved in the cellular response to stress in *S. pombe* (23). A mitogen-activated protein kinase-mediated signal transduction pathway regulates a pair of transcription factors that control the cellular response to stress, including heat shock (Fig. 5A). To test whether this pathway might be required for the Ded1 response to heat shock, strains with mutations in components of the pathway were examined for the behavior of Ded1 following transient exposure to high temperature. As shown in Fig. 5B, the additional Ded1 band is generated in response to heat shock.
The Ded1 polypeptide is modified differently in response to a variety of stress conditions. A, Ded1 does not respond to agents that damage DNA (CPT and UV), inhibit DNA replication (hydroxyurea), cause oxidative stress (H$_2$O$_2$), or change the osmolarity of the medium (KCl and sorbitol). Mid-log phase cells were exposed to the indicated stressors as described under “Experimental Procedures,” and the lysates were examined by immunoblot for Ded1. B, the Ded1 protein disappears upon depletion of nitrogen source. Both wild-type and chk1::ura4 deletion strains were cultured to mid-log phase, washed three times with medium lacking nitrogen source, resuspended in the same medium, and incubated for the times indicated. The lysates were probed for Ded1 (upper panel) and Cdc2 (lower panel). C, the Ded1 protein is modified upon depletion of carbon source in a Chk1-independent manner. The cells were cultured as in B except the medium lacked a carbon source. The lysates were probed for Ded1 (upper panel) and Cdc2 (lower panel). D, the modified forms of Ded1 generated upon depletion of carbon source or heat shock are different. The lysates prepared after depletion of carbon source or heat shock were loaded side-by-side to compare the mobility shifts caused by each treatment.

A second example indicating a connection between translational control and checkpoints comes from mammalian cells. A subtractive library approach was taken to identify protein intermediates associated with the heavy polysome fraction from cells overexpressing eIF4E, the mRNA cap recognition protein. A subunit of ribonucleotide reductase was identified in this fraction (27). Although the RNR2 protein was enriched under these conditions, the RNR2 transcript was not. Yeast cells with simultaneous mutations in the eIF4E homologue, CDC33, and RNR2 are synthetically lethal at temperatures permissive for either single mutant. These results suggest a link between translation and the regulation of nucleotide pools for DNA replication.

Our examination of the Ded1 protein suggests that the polypeptide is not modified in response to checkpoint activation caused by DNA damage or DNA replication blocks. The Ded1 protein itself is stable and unmodified throughout the vegetative cell cycle. However, exposure of cells to heat shock or removal of carbon source from the medium results in the appearance of modified forms of Ded1. The forms that appear on yeast cells to enter a stationary phase from which cells of opposite mating type can conjugate and sporulate. Between 2 and 4 h of shifting cells to nitrogen-free medium, the Ded1 protein disappears, whereas the Cdc2 protein is still present at 4 h (Fig. 6B). Interestingly, in an independent experiment, Kawamukai (13) demonstrated that the message for Ded1 is stable after 4 h in nitrogen-depleted medium. Thus, it is likely that the effect of nitrogen starvation on Ded1 stability occurs post-transcriptionally.

Carbon source depletion causes fission yeast cells to enter a stationary phase following genome duplication. Within 1 h of carbon source depletion, Ded1 is modified in such a way as to alter its mobility on SDS-PAGE (Fig. 6C). A comparison of samples prepared from carbon source-depleted and heat-shocked cells indicates that the altered mobility forms of Ded1 generated in each instance are distinct (Fig. 6D).

**DISCUSSION**

Currently there is no direct connection between the regulation of protein translation and the checkpoint response of fission yeast to DNA damage. Nonetheless, we have uncovered an interaction at two levels between the checkpoint kinase Chk1 and the DEAD box helicase, Ded1. Both Chk1 and Ded1 can suppress the inviability of a cold-sensitive loss of function allele of Cdc2. Secondly, Chk1 and Ded1 can physically interact in vivo. We were unable to detect any modification of the Ded1 protein in response to DNA damage. It is possible that the activity of Ded1 as a helicase or ATPase or in general protein translation could be affected following DNA damage. Experiments to test these possibilities could be revealing. Likewise, loss-of-function of Chk1 clearly affects the cellular response to DNA damage but does not appear to compromise the ability of cells to survive heat shock. Thus, the functional interaction between Chk1 and Ded1 remains elusive at this time.

Connections between translation and checkpoints have begun to emerge in other systems. The *Drosophila* transforming growth factor-α-related protein Gurken controls dorsoventral patterning during oogenesis (24). Expression of Gurken protein requires Vasa, the *Drosophila* homologue of the translation factor eIF4A, a DEAD box helicase related to but distinct from Ded1 (25). In response to persistent double-stranded breaks generated during meiosis, Vasa undergoes modification, and Gurken fails to accumulate (26). Both of these events require the presence of the Mei-41 protein, a member of the ATM/ATR family of checkpoint kinases. Thus, it has been proposed that Vasa responds to the meiotic checkpoint and results in decreased translation of Gurken.

To determine whether alternative forms of Ded1 appear in response to other types of cellular stress, the cells were exposed to a variety of conditions and lysates examined by immunoblot for the Ded1 protein. As shown in Fig. 6A, no response of Ded1 is detected in response to DNA damage (treatment with CPT or UV), oxidative stress (treatment with H$_2$O$_2$), or osmotic stress (KCl or sorbitol).

The behavior of Ded1 was also examined upon depletion of the nitrogen or carbon source. Nitrogen depletion causes fission shock in all strains tested, indicating that this modification does not require the function of the mitogen-activated protein kinase stress response pathway.

The lower mobility form of Ded1 could be either a newly synthesized form or a modified form of existing protein. To determine whether accumulation of the heat shock-induced form requires active translation, the cells were treated with the translation inhibitor cycloheximide prior to heat shock treatment. As shown in Fig. 5C, the appearance of the altered mobility form of Ded1 is blocked by inhibition of protein synthesis.

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SDS-PAGE after these treatments are different. The mobility of the band that appears following heat shock is more distinct from unmodified Ded1 than is the band that appears following carbon starvation (Fig. 6D). Treatment of cells with chloroamphenicol demonstrates that the appearance of the slower mobility form of Ded1 after heat shock is blocked when translation is inhibited. This result is consistent with the possibility that the slower mobility form is a new translation product. However, it is also possible that the new form is a covalently modified version of Ded1 whose modification is dependent on the activity of a short-lived protein. Alternatively, the slow mobility form may require the activity of a signaling pathway that is inhibited in the presence of cycloheximide.

Why Ded1 and Chk1 rescue the loss of function of the Cdc2-r4 cyclins Cdc13 and Cig2 (11). However, Ded1 does not appear to rescue cdc2-r4 by increasing Cdc13 levels. Nonetheless, it is possible that modestly increased levels of Ded1 up-regulate other positive regulators of cell cycle progression or down-regulate the expression of negative regulators of cell cycle progression. Future studies should reveal the mechanism through which Ded1 contributes to mitotic progression and may indicate the relevance of the physical interaction of Ded1 with Chk1.

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REFERENCES

1. Nurse, P. (1990) Nature 344, 503–508
2. Morgan, D. O. (1997) Annu. Rev. Cell Dev. Biol. 13, 261–291
3. Gould, K. L., and Nurse, P. (1989) Nature 342, 39–45
4. Lundgren, K., Walworth, N., Booher, R., Dembski, M., Kirschner, M., and Beach, D. (1991) Cell 64, 1111–1122
5. Walworth, N., Davey, S., and Beach, D. (1993) Nature 363, 368–371
6. de la Cruz, J., Kressler, D., and Linder, P. (1999) Trends Biochem. Sci. 24, 192–198
7. de la Cruz, J., Iost, I., Kressler, D., and Linder, P. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5201–5206
8. Chuang, R. Y., Weaver, P. L., Liu, Z., and Chang, T. H. (1997) Science 275, 1468–1471
9. Iost, I., Dreyfus, M., and Linder, P. (1999) J. Biol. Chem. 274, 17677–17683
10. Struhl, K. (1985) Nucleic Acids Res. 13, 8587–8601
11. Grallert, B., Kearsey, S. E., Lenhard, M., Carlson, C. R., Nurse, P., Boye, E., and Labib, K. (2000) J. Cell Sci. 113, 1447–1458
12. Forbes, K. C., Humphrey, T., and Enoch, T. (1998) Genetics 150, 1361–1375
13. Kawamura, M. (1999) Biochim. Biophys. Acta 1446, 93–101
14. Chen, L., Liu, T.-H., and Walworth, N. C. (1999) Genes Dev. 13, 675–685
15. Moreno, S., Klar, A., and Nurse, P. (1991) Methods Enzymol. 194, 795–823
16. Cottarel, G., Beach, D., and Deuschle, U. (1993) Nature 368, 945–948
17. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, p. 684, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
18. Walworth, N. C., and Bernards, R. (1996) Science 271, 353–356
19. Ayscough, K., Hayles, J., MacNeill, S. A., and Nurse, P. (1992) Mol. Gen. Genet. 232, 344–350
20. Daga, R. R., and Jimenez, J. (1999) J. Cell Sci. 112, 3137–3146
21. Wan, S., Capasso, H., and Walworth, N. C. (1999) Yeast 15, 821–828
22. Duncan, R. F. (1996) in Translational Control (Hershey, J. W. B., Mathews, M. B., and Sonenberg, N., eds) pp. 271–283, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
23. Wilkinson, M. G., and Millar, J. B. (2000) FASEB J. 14, 2147–2157
24. Ray, R. P., and Schupbach, T. (1996) Genes Dev. 10, 1711–1723
25. Styhler, S., Nakamura, A., Swan, A., Suter, B., and Lasko, P. (1998) Development 125, 1569–1578
26. Ghabrial, A., and Schupbach, T. (1999) Nature Cell Biol. 1, 354–357
27. Abd, M. R., Li, Y., Anthony, C., and DeBenedetti, A. (1999) J. Biol. Chem. 274, 35991–35998
28. Shieh, J. C., Wilkinson, M. G., and Millar, J. B. A. (1998) Mol. Biol. Cell 9, 311–322
