Budding Yeast CTDK-I Is Required for DNA Damage-Induced Transcription

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CTDK-I phosphorylates the C-terminal domain (CTD) of the large subunit of yeast RNA polymerase II in a reaction that stimulates transcription elongation. Mutations in CTDK-I subunits—Ctk1p, Ctk2p, and Ctk3p—confer conditional phenotypes. In this study, we examined the role of CTDK-I in the DNA damage response. We found that mutation of individual CTDK-I subunits rendered yeast sensitive to hydroxyurea (HU) and UV irradiation. Treatment with DNA-damaging agents increased phosphorylation of Ser2 within the CTD repeats in wild-type but not in ctk1Δ mutant cells. Using microarray hybridization, we identified genes whose transcription following DNA damage is Ctk1p dependent, including several DNA repair and stress response genes. Following HU treatment, the level of Ser2-phosphorylated RNA polymerase II increased both globally and on the CTDK-I-regulated genes. The pleiotropic phenotypes of ctk mutants suggest that CTDK-I activity is essential during large-scale transcriptional repatterning under stress and unfavorable growth conditions.

The large subunit of RNA polymerase II contains a conserved C-terminal domain (CTD) composed of a heptapeptide motif, YSPTSPS, repeated 26 to 52 times in different species (7). The CTD plays an important role in transcription, as deletions or mutations within the repeat lead to conditional phenotypes and loss of viability (30). The CTD is phosphorylated during transcription initiation at two serine residues, Ser2 and Ser5. Both Ser residues are essential for viability, though their functions have been separated genetically and biochemically (21, 34, 44). Cross-linking experiments have revealed that phosphorylation of Ser5 occurs during transcription initiation, whereas phospho-Ser2 is associated with elongating polymerases (21). CTD phosphorylation regulates the interaction of the polymerase with components of the preinitiation complex, transcription elongation factors, and RNA processing factors (5, 28, 32, 42).

Extensive biochemical analyses have identified numerous protein kinases specific for the CTD. In Saccharomyces cerevisiae, Ctk1p and Srb10p phosphorylate Ser2; Kin28p, Srb10p, and Bur1p phosphorylate Ser5 (2, 6, 11, 15, 29). The functional outputs of these events vary: Kin28p is required for transcription initiation, whereas phosphorylation by Srb10p inhibits transcription (15). Ctk1p assembles with Ctk2p, a cyclin-like protein, and Ctk3p into a three-subunit complex, CTDK-I (40). The purified complex increases the rate of transcription elongation in vitro (26). The CTK genes are not essential for viability, but mutants exhibit slow-growth and cold-sensitive phenotypes (25). Interestingly, environmental signals can influence the extent of Ser2 phosphorylation, which increases when yeast cells prepare to enter stationary phase (34). This increase is mediated by Ctk1p and is thought to activate genes in stationary phase (33). The Fcp1p phosphatase dephosphorylates phospho-Ser2 at the end of the transcription cycle (4, 20).

Yeast cells exposed to diverse environmental conditions adjust their transcription program by coordinate activation and repression of multiple genes. For example, exposure of cells to genotoxic agents leads to induction of DNA repair and environmental stress response genes (12, 18). Interestingly, the transcription profile induced by DNA damage overlaps with that caused by transition into stationary phase (17). In this study, we observed that mutation of CTK genes rendered yeast cells sensitive to UV irradiation and hydroxyurea (HU). We found that DNA-damaging agents increased phosphorylation of the CTD on Ser2 in a Ctk1p-dependent manner. Using microarray hybridization, we identified genes regulated by Ctk1p. Several of these Ctk1-dependent genes are required for DNA repair and environmental stress responses.

MATERIALS AND METHODS

Yeast strains and growth conditions. Yeast strains were derivatives of W303a (ade2-1 thr1-1 leu2-3,112 his3-11,15 ura3-1) (36). Yeast transformations were performed as described previously (13). Yeast cultures were grown in yeast extract-peptone-dextrose (YPD) medium and in synthetic complete (SC) medium lacking individual amino acids. Cells were treated with DNA-damaging agents in YPD medium in the presence of 0.03% methylmethane sulfonate (MMS) or 0.1 M HU for the times indicated in the figure legends. To impose mild amino acid starvation, cells grown in SC medium (optical density at 600 nm [OD600] ~0.5) were collected by centrifugation and inoculated at the same density into synthetic minimal medium lacking most amino acids and adenine (YNB-AA, 2% glucose, 20 mg each of uracil, histidine, tryptophan, and leucine per liter).

The ctk1Δ strain (W303a ctk1Δ::HIS3) was a kind gift of Opher Gileadi (Weizmann Institute of Science, Rehovot, Israel) (19). A functional copy of CTKI was a kind gift of Arno Greenleaf (Duke University, Durham, N.C.) (25). The CTKI coding sequence was reamplified and hemagglutinin (HA) tagged by PCR with primers MSO776 (5′-GGGGATCCATGTCCTACAATAATGGC-3′) and MSO778 (5′-GACGTCATATGGATAGGCGCCTTTATCATCATCGTCATTATT-3′) (underlined residues correspond to introduced restriction sites). All PCR products were

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were sequenced to verify that no additional mutations were introduced. The amplified fragment was inserted between the BamHI and PstI sites in YCplac22 (14), yielding YCplac22-CTK1-HA, which rescued the cold-sensitive phenotype of ctk1A cells.

Deletion of ctk1 (W303a ctk3::URA3) was achieved by transforming ctk3Á- Yplac211 into W303a, ctk2Á-Yplac211 contained 200 bp from the noncoding 5’ end and 200 bp from the noncoding 3’ end of CTK2 to replace the genomic copy of CTK2 with URA3. The resulting URA4+ clones demonstrated a cold-sensitive phenotype. Gene disruption was verified by PCR. CTK2 was tagged with the HA epitope by PCR amplification with primers MSO862 (5’-CTCGTTCGCAAT CATTCTGGAG; MSO862) and MSO857 (5’-AGAGCCCTTAGTCGAAC-3’; MSO857) to demonstrate the minimal number of clusters with fundamentally different patterns. Annotations for gene function were from the Yeast Proteome Database. The data represent background-corrected log2 values of the red/green ratio measured for each gene. A full set of genes and their fold changes in text table-delimited format are available from the authors.

**Chromatin immunoprecipitation.** Chromatin cross-linking and immunoprecipitation were performed as previously described (21, 24, 39, 41). Briefly, 100-ml cultures (OD600 ~0.8) were treated with 1% formaldehyde for 15 min at 23°C and quenched by addition of 240 mM glycine for 5 min at 23°C. Cells were collected and washed twice with ice-cold TBS. Cells were lysed by shaking yeast suspensions with 0.5 g of glass beads (diameter, 0.5 mm; Sigma) in a bead beater (Biospec Products) for five 30-s pulses with 30 s on ice between pulses. Glass beads and cell debris were removed by centrifugation at 66,000 rpm in a Microfuge at 4°C for 10 min. The supernatant was clarified by centrifugation at 65,000 rpm in a TLA 100.2 rotor (Beckman) for 10 min at 4°C. Protein concentrations were determined by using the Bradford assay (Bio-Rad) with bovine serum albumin (BSA) as a standard. Yeast extracts were aliquoted, frozen in liquid nitrogen, and stored at −80°C.

For analysis of RNA polymerase, 5 μg of yeast protein extracts was separated on sodium dodecyl sulfate (SDS)-polyacrylamide gels (6% total acrylamide) and transferred to Immobilon-P membranes (Millipore). To detect phosphospecific isoforms, membranes were first incubated with antibody H5 or H14 (both from Covance) at 1 μg/ml in TBST-BSA (TBST-0.1% Tween 20–5% BSA) overnight at 4°C and then probed with anti-mouse immunoglobulin M (IgM) coupled to horseradish peroxidase (500 ng/ml; Pierce). To detect unphosphorylated polyamines with 10 mM Tris-Cl (pH 8.0), 10 μg each of leupeptin, chymostatin, and pepstatin (Chemicon) per ml, 1 mM dithiothreitol (DTT) for 1 h at 65°C with brief vortexing every 10 min. Aqueous phases were extracted with phenol-chloroform-isoamyl alcohol (25:24:1) and chloroform-isooamy alcohol (24:1). RNA was ethanol precipitated for 3 h at −20°C, centrifuged at 10,000 rpm for 15 min at 4°C in an SS34 rotor (Sorval), washed with 70% ethanol, and resuspended in 300 μl of H2O treated with diethyl procarbamate (Sigma). The RNA concentration was determined spectrophotometrically.

**Northern blot hybridization.** For hybridization analysis, 20 μg of RNA was electrophoresed on 1% formaldehyde-agarose gels and transferred to GeneScreen membranes (NEN Life Science Products), as described previously (37). The membranes were probed with short DNA fragments labeled with [α-32P]dCTP by using a random priming kit (Roche). Hybridizations were carried out for 10 h at 42°C in 5× SSPE (1× SSPE = 0.18 M NaCl, 10 mM NaH2PO4, 1× EDTA) at pH 7.5. Chromosome V intergenic primers were located between chromosome V and 0.1% SDS; 10 mM EDTA, 0.1% sodium deoxycholate, 0.1% SDS, 10 μg each of leupeptin, chymostatin, and pepstatin (Chemicon) per ml, 0.5% formamide, 5× SSPE (1× SSPE = 0.15 M NaCl plus 0.015 M sodium citrate), 0.1% SDS–5 μg of poly(A) (Sigma) per ml, denaturated at 94°C for 4 min, and spotted onto a custom-made yeast whole-genome microarray (8). Hybridization was carried out in a CMT hybridization chamber (Corning) at 50°C for 10 h. The microarray was washed sequentially with 100 ml of 2× SSC–0.1% SDS at 42°C for 1 min, 0.1× SSC–0.1% SDS at 23°C for 5 min (twice), and 0.1× SSC at 23°C for 2 min (twice). The microarray was dried in a stream of nitrogen and read on a GenomicArray scanner (Axon Instruments Inc.).

The fold change for every gene was calculated as an average of pixel-by-pixel ratios by using GenePix Pro software (Axon Instruments Inc.). Data shown represent means from at least two hybridization experiments, including swapping of Cy3 and Cy5 probes. Clustering analysis was performed with the Cluster and Treeview programs developed by Michael Eisen, Lawrence Berkeley National Lab. (available at http://rana.stanford.edu/software). We used K-means clustering to demonstrate the minimal number of clusters with fundamentally different patterns. Annotations for gene function were from the Yeast Proteome Database. The data represent background-corrected log2 values of the red/green ratio measured for each gene. A full set of genes and their fold changes in text table-delimited format are available from the authors.

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For chromatin immunoprecipitation, 50 μl of protein A Dynabeads (Dynal) was coupled to 25 μg of a monoclonal antibody against RNA polymerase II (H5 or H14; Covance). The beads were incubated with 100 μl of yeast extracts in 400 μl of FA buffer for 3 h at 4°C. Bead-protein complexes were washed twice for 7 min with 1 ml of FA buffer, twice with FA buffer containing 500 mM NaCl, once with 10 mM Tris–pH 8.0–1 mM EDTA–250 mM LiCl–0.5% NP-40, and once with 10 mM Tris–pH 8.0–1 mM EDTA, by using a magnetic particle concentrator (Dynal). To elute precipitated material, the beads were heated in 400 μl of 25 mM Tris–pH 7.5–10 mM EDTA–0.5% SDS at 65°C for 10 min. Formaldehyde cross-links were reversed by incubation with 20 μg of proteinase K (Roche) for 1 h at 42°C, followed by 5 h at 65°C. The eluted DNA was purified with phenol-chloroform, precipitated with ethanol, dissolved in 400 μl of 10 mM Tris–pH 8.0–1 mM EDTA, and stored at −20°C.

For quantitative PCR, 1 μl of the immunoprecipitated DNA or 0.01 μl of the input material was used. PCR mixtures (25 μl) contained 0.5 μM primers, 0.1 mM deoxyxynucleoside triphosphates, 2 μl of [α-32P]dCTP (3,000 Ci mmol−1), 1× PCR buffer, and 0.5 μl of Taq polymerase (Roche). Cycling was performed for 20 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C. PCR products were resolved on 7% polyacrylamide–Tris-borate-EDTA gels and visualized by autoradiography.

Primers were 24- to 26-mers designed to amplify DNA fragments within the promoter region and immediately downstream of the transcription start site. Numbers in primer designations are relative to the gene coding sequence. The coordinates for the probes were located at 0 and 5′ of YEL072W. Primer sequences were as follows: FLRJ_1125-5′-ATGGCGGCGAT AATTGTCAGGTA; FLRJ_1124-5′-TTCTGGCTTCACTTCCACGGC GAC;
RESULTS

Mutations of CTK genes render yeast sensitive to DNA damage. Genes encoding subunits of CTDK-I are not essential for viability, but mutants exhibit growth-impaired cold-sensitive phenotypes (40). In an attempt to identify additional roles for CTDK-I, we examined the viability of ctk mutants under various growth conditions. We found that yeast strains carrying deletions of individual CTK genes were unable to grow in the presence of the DNA synthesis inhibitor HU (Fig. 1). Deletions of CTK1, CTK2, or CTK3 produced similar phenotypes, consistent with a common function within CTDK-I. Plasmids carrying functional copies of the disrupted genes complemented the HU sensitivity. These ctk mutants were also sensitive to UV irradiation (Fig. 1) and slightly sensitive to MMS (data not shown). A single amino acid substitution within the Ctk1p catalytic domain, Asp324 to Asn324, rendered yeast sensitive to both HU and UV irradiation, indicating that protein kinase activity is essential for this function (data not shown). Therefore, it appears that CTDK-I has a specialized role in the DNA damage response.

Phosphorylation of the CTD at Ser2 increases after DNA damage. The sensitivity of ctk cells to genotoxic agents suggested that CTD phosphorylation is required for the DNA damage response. To test whether DNA damage alters RNA polymerase II phosphorylation, wild-type cells were treated with HU for different times, and resulting protein extracts were compared. Various CTD isoforms were detected by using monoclonal antibodies that recognize phospho-Ser2, phospho-Ser5, and unphosphorylated polymerase. As seen in Fig. 2A, HU treatment increased phosphorylation of Ser2, whereas levels of phospho-Ser5 and unphosphorylated polymerase were unchanged. Levels of phospho-Ser2 remained high during the course of the experiment, consistent with the continued presence of the DNA-damaging agent. We observed similar changes in CTD phosphorylation after treatment of yeast with MMS (data not shown), indicating that this effect was general for different types of DNA damage.

Since Ctk1p is known to phosphorylate Ser2, we were interested in whether Ctk1p is responsible for the increased CTD phosphorylation. To this end, we compared phosphorylation of RNA polymerase II in HU-treated wild-type and ctk1Δ cells. Figure 2B shows that deletion of ctk1 abolished Ser2 phosphorylation in untreated cells (Fig. 2B, lane 5) and following HU treatment (Fig. 2B, lanes 6 to 8). The apparent phosphorylation of Ser5 increased at all times in ctk1Δ cells, possibly due to better accessibility of monoclonal antibodies to Ser5 in the absence of Ser2 phosphorylation. Therefore, CTDK-I is responsible for the DNA damage-induced hyperphosphorylation of RNA polymerase on Ser2.

CTDK-I modulates DNA damage-induced expression of
mutants might be due to a transcriptional de
Because various types of DNA lesions induce transcription of
genes required for protection from DNA-damaging agents.
we
RNR
genes.

We hypothesized that the conditional phenotype of
ctk mutants might be due to a transcriptional deficiency of
genes required for protection from DNA-damaging agents.
Because various types of DNA lesions induce transcription of
RNR genes, which encode subunits of ribonucleotide reductase
(10), we first examined RNR expression in a ctk background.
As seen in Fig. 3, transcription of RNR1 was reduced in ctk1Δ
cells and its induction by HU and MMS was attenuated (Fig. 3,
lanes 2, 5, and 8). Activation of RNR2 and RNR3 was also
muted in ctk1Δ cells, though to a lesser extent than that of
RNR1. A tagged version of CTK1 introduced in ctk1Δ cells
restored expression of RNR genes (Fig. 3, lanes 3, 6, and 9).

The amount of RNR1 mRNA can be important for cell
survival, as its overexpression, even by twofold, rescues the
lethality of mecl and rad53 DNA damage checkpoint mutants
(9). We reasoned that if RNR1 is a major target of Ctk1p, its
overexpression might rescue the ctk1 phenotype. Therefore,
ctk1Δ cells were transformed with a multicopy plasmid carrying
RNR1 under the control of a constitutive GAP promoter. The
resulting strain demonstrated the same level of HU sensitivity
as the original mutant, indicating that RNR1 is not the only
important gene dependent on CTDK-I (data not shown).

The transcriptional response to HU treatment requires Ctk1p.
To identify other genes regulated by Ctk1p, we set up
microarray hybridization experiments. Recent work has dem-
Onstrated that MMS treatment changes the transcriptional
profiles of many yeast genes (12, 18). We extended this analysis
to HU because ctk mutants have a stronger phenotype in HU.
Five sets of hybridizations were performed. In the first two,
mRNAs from wild-type cells after 30- and 60-min incubations
with HU were isolated, fluorescently labeled, mixed with con-
trol mRNA from untreated wild-type cells labeled with a dif-
ferent fluorophore, and hybridized to yeast microarrays. The
abundance of every transcript in the HU-treated sample was
calculated relative to that in the untreated control sample (Fig.
4A, arrays 1 and 2). In the third experiment, mRNA levels in
untreated ctk1Δ cells were compared to those in untreated
wild-type cells (Fig. 4A, array 3). The final two experiments
compared mRNA levels in HU-treated ctk1Δ cells to those in
HU-treated wild-type cells (Fig. 4A, arrays 4 and 5). The av-
erage data from the microarray hybridizations were analyzed
by K-mean clustering, so that genes with similar expression
patterns were grouped together. Only results for the 187 genes
whose expression changed at least threefold in at least one
experiment are shown.

Five clusters of gene expression patterns were evident (Fig.
4A). Cluster I comprises genes whose expression was induced
to similar extents by HU treatment of wild-type cells and ctk1Δ
cells. Genes in this cluster include many involved in stress
responses, such as HSP26, SSE2, and TRX2, and some with
functions in carbohydrate metabolism, such as GLK1, PGM2,
and TPS1. Cluster II comprises genes whose expression was
repressed in both wild-type cells and ctk1Δ cells. Several of
these genes, such as FAL1, NOP2, and NBSR, are involved in
aspects of RNA processing. These two clusters demonstrate
that not all changes in gene expression following HU treatment
require CTK1.

The expression of 108 genes was altered in untreated and
treated ctk1Δ cells (Fig. 4A, array 3, clusters III, IV, and V).
Cluster IV comprises genes whose transcription levels in-
creased in ctk1Δ cells, whether or not those cells were treated
with HU. HU treatment caused a very slight overall stimula-
tion of these genes in wild-type cells. Most of the genes in this
cluster, such as LYS1, ARG1, and HIS5, encode amino acid
biosynthesis enzymes. In contrast, cluster V comprises genes
that were inhibited in ctk1Δ cells, again whether or not those
cells were treated with HU. HU treatment caused a very slight
overall inhibition of these genes in wild-type cells.

We were particularly interested in genes showing coordinate
induction by HU in wild-type cells but reduced expression in
HU-treated ctk1Δ cells (Fig. 4A, cluster III, and Fig. 4B).
Several of these transcripts encode proteins directly involved in
DNA repair, including the protein kinase Dunlp, the ribonu-
ucleotide reductase Rnr2p, and the checkpoint protein Hug1p.
A recent study demonstrated that these genes are specifically
induced by various types of DNA damage (12). Although
RNR1 is induced by HU (Fig. 3), its absence from the microar-
ray display is probably due to the absence of a corresponding
DNA feature (altogether, about 50 genes were found to be
missing from the microarray).

Products of other genes in cluster II participate in different
aspects of detoxification, including the glutathione transferase
Gtt2p, the flucunazole resistance protein Flr1p, and a protein
of unknown function, YNL134C. Apparently, functions of
these proteins provide a broad spectrum of defense mecha-
nisms utilized by cells to neutralize the toxic effects of HU.
FIG. 4. Transcriptional responses of wild-type (WT) and ctk1Δ cells to HU. (A) Results of five experiments (numbered on the right) are shown. Experiments 1 and 2 compared mRNA levels in wild-type cells treated with HU for 30 and 60 min, respectively, to those in untreated wild-type cells. Experiment 3 compared mRNA levels in untreated ctk1Δ cells to those in untreated wild-type cells. Experiments 4 and 5 compared mRNA levels in HU-treated ctk1Δ cells to those in HU-treated wild-type cells after 30 and 60 min of treatment, respectively. Only the 187 genes whose expression changed at least threefold in at least one experiment were included in the K-mean clustering. The color scale at the bottom represents the fold change in transcript abundance; red indicates gene induction by HU, and green indicates gene repression. (B) Genes activated by HU in wild-type cells but not in ctk1Δ cells (cluster III). Only the subset of genes with known functions is shown. Annotations for gene functions are from the Yeast Proteome Database. The full set of 187 genes and their fold changes are available from the authors.
Although their expression is compromised in untreated ctk1Δ cells, their activation appears to become vital only under the stress of HU treatment. The collective abnormal expression of many DNA repair and detoxification genes probably explains the HU-sensitive phenotype of ctk1Δ cells.

The requirement for Ctk1p is promoter and pathway specific. We were interested in confirming the microarray results, in determining whether different DNA-damaging agents induced the same genes, and in determining whether all three CTDK-I subunits are necessary for this induction. To this end, ctk1Δ, ctk2Δ, and ctk3Δ cells were treated with DNA-damaging agents, and the abundances of several mRNAs were examined by Northern blot hybridization. As expected, MMS and HU treatments induced transcription of FLR1, GTT2, and YNL134C in wild-type cells, though the extent of stimulation varied greatly (Fig. 5A, lanes 1 to 3). Mutation of CTDK-I subunits disrupted activation of target genes, indicating that all three Ctk proteins were required for transcriptional induction (Fig. 5A, lanes 4 to 12). Interestingly, MMS and HU had different stimulatory effects on the expression of these genes. For instance, FLR1 was hardly induced by MMS treatment, whereas YNL134C was induced equally by MMS and HU (Fig. 5A, lanes 1 to 3). In general, disruption of CTDK-I had a greater effect on HU-induced transcription than on MMS-induced transcription, perhaps reflecting the selection of these genes as strongly HU inducible in the microarray experiment. The differential effects of ctk mutants on various genes indicate that reduced expression was not due to a general block to induced transcription but rather was dependent on the specific signaling pathway regulating a given promoter (Fig. 4A, clusters I and II versus clusters III, IV, and V; Fig. 5A). Thus, it is likely that activation of GTT2 by MMS and HU occurs through different mechanisms and may involve distinct sets of transcription factors. The nature of these factors would dictate their requirement for Ctk1p kinase activity and CTD phosphorylation. Interestingly, ctk mutations had little effect on transcription of these genes in untreated cells, implicating CTDK-I in the global repatterning of expression in HU-treated cells.

We wondered whether the attenuated expression of stress response genes was due to a delay in transcriptional activation, in which case their expression would increase after prolonged exposure to genotoxic agents. We addressed this question with a time course experiment in which cells were incubated with HU for different times. In wild-type cells, levels of the FLR1 and GTT2 transcripts gradually increased, consistent with the continued presence of DNA damage (Fig. 5B, lanes 1 to 5). In contrast, transcription of these genes remained at undetectably low levels in ctk1Δ cells (Fig. 5B, lanes 6 to 10). Therefore, it seems unlikely that attenuated expression was due to a slow rate of transcriptional activation.

HU treatment increases binding of phosphorylated polymerase to induced genes. Since treatment of cells with HU increased phosphorylation of the CTD on Ser2 (Fig. 2), we wondered whether induced genes would show an increase in Ser2 phosphorylation of bound polymerase. To address this question, we used chromatin immunoprecipitations after light formaldehyde treatment to examine the levels of phosphorylated polymerase associated with selected genes. We found that HU treatment increased cross-linking of phosphorylated polymerase to FLR1, GTT2, and YNL134C, which correlated with the transcriptional induction of these genes, but not to ADH1, which is expressed constitutively (Fig. 6, lanes 1, 2, 6, and 7). In untreated cells, polymerase cross-linking to transcriptionally inactive genes was relatively low and comparable to cross-linking at intergenic regions, which served as internal controls for nonspecific binding. Deletion of CTK1 decreased cross-linking of phospho-Ser2 polymerase for all HU-induced genes, confirming that CTDK-I is the major kinase responsible

![Figure 5](image-url)
for Ser2 phosphorylation (Fig. 6, lanes 3 and 4; see also Fig. 2). Cross-linking of phospho-Ser2 polymerase to ADH1 was reduced but not eliminated in ctk1Δ cells; presumably the residual phosphorylation is carried out by Srb10p. The reduction of Ser2 phosphorylation in ctk1Δ cells also decreased the cross-linking of phospho-Ser5 polymerase. Therefore, HU treatment increased the phosphorylation of polymerase on transcriptionally induced genes. It is possible that some genes, such as FLR1, GTT2, and YNL134C, are intrinsically sensitive to the level of polymerase phosphorylation. In the absence of phospho-Ser2, as observed in ctk1Δ cells, transcription initiation or elongation from their promoters might be impaired.

**Ctk1p is required for reprogramming of gene expression upon amino acid starvation.** We wondered whether CTDK-I function is restricted to DNA damage signaling or whether it is required for adjustments to other environmental changes. Therefore, we examined the transcriptional response to amino acid starvation, a nontoxic perturbation often encountered by yeast cells under natural conditions. In this experiment, cells were shifted from a rich medium to a minimal medium depleted of all amino acids not required for growth of this strain. Five sets of hybridizations were performed. In the first two, wild-type cells starved for amino acids were compared to untreated wild-type cells (Fig. 7A, arrays 1 and 2). The third hybridization examined ctk1Δ and wild-type cells in a rich medium. The last two hybridizations compared mRNAs from ctk1Δ and wild-type cells following amino acid starvation (Fig. 7A, arrays 4 and 5).

As expected, depletion of amino acids activated transcription of at least 180 genes involved in amino acid biosynthesis pathways and general stress responses. Approximately half of these genes were similarly induced in wild-type and ctk1Δ cells, indicating that signaling of amino acid starvation was not impaired by the ctk1Δ mutation (Fig. 7A, cluster I). Cluster III comprises 94 genes whose transcription was induced in wild-type cells but not in ctk1Δ cells. This group includes genes encoding enzymes involved in amino acid biosynthesis, such as *LYS9*, *URA10*, and *PUT4*, and stress response genes *GRE2*, *YNL134C*, and *YDR533C*, which had previously been implicated in HU response (Fig. 7B). Therefore, it appears that Ctk1p is required for global reprogramming of gene transcription in response to amino acid starvation and, presumably, other environmental stresses.

**DISCUSSION**

Numerous studies indicate that phosphorylation of the C-terminal domain of the large subunit of RNA polymerase II on Ser2 and Ser5 within the heptapeptide repeats regulates transcription initiation, elongation, and RNA processing (3, 21, 31, 42). Several lines of evidence suggest that Ser2 may have an additional function, since its phosphorylation increases when yeast cells approach stationary phase and after heat shock (34). The results of this study indicate that Ser2 phosphorylation also plays a role in the DNA damage response following HU and MMS treatments.

Given the multitude of CTD functions, it is not surprising that several enzymes regulate its phosphorylation. Ser5 is phosphorylated by Kin28p, Srb10p, and Bur1p (11, 15, 29). Srb10p can also phosphorylate Ser2 (2). However, deletion of *CTK1* or any other subunit of the CTDK-I complex dramatically decreased Ser2 phosphorylation in vivo, indicating that Ctk1p is the major protein kinase responsible for this phosphorylation (4, 33) (Fig. 2B). Yeast cells carrying mutations in *CTK* genes grow like wild-type cells but are inviable at reduced temperatures and exhibit a delay during exit from stationary phase (25). In addition, we present evidence that *ctk* mutant cells are sensitive to DNA-damaging agents and UV irradiation. These pleiotropic phenotypes suggest that CTDK-I activity is essential during large-scale transcriptional repatterning under stress and unfavorable growth conditions. This hypothesis is supported by the finding that Ctk1p is required for gene activation following a nutritional shift.
Ctk1p has been implicated in transcriptional elongation. The purified CTDK-I complex stimulates the rate of transcript elongation in vitro, and the ctk1Δ mutation is synthetically lethal with mutations in transcription elongation factors such as Elp1p, Spt5p, and Ppr2p (TFIIS) (19, 26, 27). It is not known whether most or just a few genes require CTDK-I for efficient elongation. The results of this study indicate that a subset of genes fails to be induced in the absence of CTK1 (Fig. 4 and 7, clusters III and V). Some of these genes are also deregulated by elp mutants that compromise the activity of the Elongator complex (22). Because the complex binds to phosphorylated RNA polymerase, it is not surprising that CTDK-I and Elongator regulate overlapping sets of genes (32). Alternatively, some genes might be affected indirectly, due to deregulation of other transcription factors. For example, expression of ADA2, which encodes a subunit of the nucleosomal histone acetyltransferase complexes ADA and SAGA (1, 35), was compromised in ctk1Δ cells.

We noticed that the abundances of several mRNAs increased in ctk1Δ cells, suggesting that CTDK-I might function as a gene-specific repressor. Repressor activity has been demonstrated previously for another cyclin-dependent kinase, Srb10p, which phosphorylates the CTD on Ser2 and Ser5 (16, 23). When this phosphorylation occurs prior to formation of the preinitiation complex, it inhibits transcription (15). Similarly, Ctk1p-mediated phosphorylation of Ser2 might have both positive and negative effects, depending on the timing and promoter context. It is also possible that some genes are induced indirectly, in compensation for the loss of Ctk1p activity.

Given that ctk1Δ cells are viable, it is likely that under normal conditions, yeast cells can tolerate a great reduction in Ser2 phosphorylation. However, high-efficiency transcription may become essential under stress conditions. We showed that induction of several genes was critically dependent on the activity of CTDK-I. Deletion of CTK1 attenuated the DNA damage-induced expression of RNR1, RNR2, and RNR3, which

![Figure 7](image-url)

**FIG. 7.** Transcriptional response of wild-type (WT) and ctk1Δ cells to amino acid starvation. (A) Results of five experiments (numbered on the right) are shown. Experiments 1 and 2 compared mRNA levels in wild-type cells depleted of amino acids for 60 or 120 min, respectively, to those in wild-type cells maintained in a rich medium. Experiment 3 compared mRNA levels in ctk1Δ cells and wild-type cells in a rich medium. Experiments 4 and 5 compared mRNA levels in amino acid-depleted ctk1Δ cells to those in wild-type cells depleted of amino acids for 60 and 120 min, respectively. Only the 328 genes whose expression levels changed at least threefold in at least one experiment were included in the K-mean clustering. Roman numerals indicate five major gene clusters with similar expression patterns. Colors indicate fold changes in transcript abundance (see the color scale at bottom right). (B) Genes activated by amino acid starvation in wild-type cells but not in ctk1Δ cells (cluster III). Only the subset of genes strongly dependent on Ctk1p is shown. Annotations for gene functions are from the Yeast Proteome Database. The full set of 328 genes and their fold changes are available from the authors.
is essential for DNA repair. This finding supports previous work that implicated CTD phosphorylation in regulation of RNR transcription (43). In addition to RNR genes, several stress response genes, such as FLR1, GT22, and YNL134C, were not expressed in the absence of Ser2 phosphorylation. It appears that the collective loss of function of these genes compromises cellular defense mechanisms against DNA-damaging agents, making ctk cells sensitive to DNA damage.

Interestingly, the essential role of CTDK-I is probably not limited to the DNA damage response, as several genes were not activated in ctkΔ cells during amino acid starvation. It is likely that Ser2 phosphorylation is essential during the global reprogramming of transcription in response to various stresses, such as survival at low temperature, stationary phase, or DNA damage. Rapid gene activation requires the integrity of the general transcription machinery, which might explain why mutation of Ctk1p reduces the ability of cells to respond to a wide range of conditions.

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