environmental microbiology

Environmental Microbiology (2019) **00**(00), 00–00 doi:10.1111/1462-2920.14821

The *Neurospora* RNA polymerase II kinase CTK negatively regulates catalase expression in a chromatin context-dependent manner

Jiabin Duan,[1‡] Qingqing Liu,[1‡] Sodgerel Su,[1] Joonseok Cha,[2] Yike Zhou,[1] Ruiqi Tang,[1] Xiao Liu,[3] Ying Wang,[1] Yi Liu[2] and Qun He[1*]

[1]*State Key Laboratory of Agrobiotechnology and MOA Key Laboratory of Soil Microbiology, College of Biological Sciences, China Agricultural University, Beijing, 100193, China.*

[2]*Department of Physiology, The University of Texas Southwestern Medical Center, Dallas, Texas, 75390, USA.*

[3]*State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, China.*

Summary

Clearance and adaptation to reactive oxygen species (ROS) are crucial for cell survival. As in other eukaryotes, the *Neurospora* catalases are the main enzymes responsible for ROS clearance and their expression are tightly regulated by the growth and environmental conditions. The RNA polymerase II carboxyl terminal domain (RNAPII CTD) kinase complex (CTK complex) is known as a positive elongation factor for many inducible genes by releasing paused RNAPII near the transcription start site and promoting transcription elongation. However, here we show that deletion of CTK complex components in *Neurospora* led to high CAT-3 expression level and resistance to H_2O_2-induced ROS stress. The catalytic activity of CTK-1 is required for such a response. On the other hand, CTK-1 overexpression led to decreased expression of CAT-3. ChIP assays shows that CTK-1 phosphorylates the RNAPII CTD at Ser2 residues in the *cat-3* ORF region during transcription elongation and deletion of CTK-1 led to dramatic decreases of SET-2 recruitment and H3K36me3 modification. As a result, histones at the *cat-3* locus become hyperacetylated to promote its transcription. Together, these results demonstrate that the CTK complex is negative regulator of *cat-3* expression by affecting its chromatin structure.

Received 1 July, 2019; revised 25 September, 2019; accepted 2 October, 2019. *For correspondence. E-mail qunhe@cau.edu.cn; Tel. 8610-62731206; Fax: 8610-62731206. ‡These authors contributed equally to this work

Introduction

Molecular oxygen (O_2), as a premiere biological electron acceptor, plays an essential role in fundamental cellular functions of aerobic organisms (Scandalios, 2005). It is beneficial to the cellular aerobic metabolism and provides much higher energy yield compared with anaerobic respiration (Scandalios, 1997). However, reactive oxygen species (ROS) are generated during this physiological processes, such as the superoxide radical ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide (H_2O_2) and sometimes reactive singlet form of oxygen ($^1O_2$) (Ames *et al*., 1993; Scandalios, 2002). These intermediates can cause harm to the macromolecules in cells, such as extensive oxidation damage to DNA, unsaturated fatty acids, and protein amino acid residues (Scandalios, 2005; Schumacker, 2006; Waris and Ahsan, 2006; Glorieux *et al*., 2015). Furthermore, the oxidative stress induced by excessive ROS also acts as a mediator of apoptosis and causes various chronic conditions (Buttke and Sandstrom, 1994; Waris and Ahsan, 2006).

To relieve oxidative stress, organisms developed clearance systems which convert harmful ROS into harmless constituents. Three main types of enzymes are involved in the ROS clearance systems, superoxide dismutases, catalases (CAT) and peroxidases (McCord and Fridovich, 1969; Fridovich, 1995; Scandalios, 1997, 2002). These clearance mechanisms are highly conserved from bacteria to mammals (Zamocky *et al*., 2008). In the filamentous fungus *Neurospora crassa*, there are four *catalase* genes, *cat-1*, *cat-2*, *cat-3* and *cct-1/cat-4* (Chary and Natvig, 1989; Borkovich *et al*., 2004; Schliebs *et al*., 2006; Yamashita *et al*., 2008). CAT-1 and CAT-3 are two large monofunctional CAT, which mainly exert their catalase activities on the conidia and mycelia respectively (Hansberg *et al*., 1993; Michán *et al*., 2002). CAT-3 is expressed during late exponential growth and is

rigorously regulated by the growth conditions and environment stimulations. The *cat-3* gene is located in the right arms of chromosomes III (Chary and Natvig, 1989). Our previous study showed that 5-kb upstream region of *cat-3* gene formed a heterochromatic domain which plays a repressive role in *cat-3* expression (Wang *et al*., 2016). Furthermore, CPC1, the homologue of *Saccharomyces cerevisiae* GCN4, is an activator of *cat-3* transcription, via directly binding to the *cat-3* locus in response to developmental and oxidative stress signals (Dong *et al*., 2018; Qi *et al*., 2018). These results suggested that chromatin structure and histone modifications play major roles in regulating the inducible expression of the *cat-3* gene.

A key element of transcription regulation by RNA polymerase II is the modification on $Y_1S_2P_3T_4S_5P_6S_7$ repeats of the carboxyl terminal domain (CTD) of Rpb1, the largest subunit of RNAPII (Eick and Geyer, 2013; Stump and Ostrozhynska, 2013; Bowman and Kelly, 2014). The serine 5 and serine 2 phosphorylation (Ser5-P and Ser2-P) of the CTD are the best studied and the most conserved marks of transcription in eukaryotes. The Ser5-P is stimulated by Kin28/CDK7 (Kin28 in *S. cerevisiae* and CDK7 in mammals) (Srivastava and Ahn, 2015). There are two Ser2-P kinases, Bur1/CDK9 (Bur1 in *S. cerevisiae* and CDK9 in mammals) and Ctk1/CDK12 (Ctk1 in *S. cerevisiae* and CDK12 in mammals) (Bowman and Kelly, 2014). During transcriptional initiation, general transcription factors and a mediator complex are recruited to the promoter of genes by transcription activators; these events are responsible for RNAPII recruitment (Thomas and Chiang, 2006; Srivastava and Ahn, 2015). Furthermore, Ser5 is phosphorylated by Kin 28/CDK7, which stimulates RNAPII for promoter clearance and functions in pre-mRNA 5′-capping and initiation–elongation transition (Rodriguez *et al*., 2000; Sogaard and Svejstrup, 2007; Viladevall *et al*., 2009; Srivastava and Ahn, 2015). Afterwards, the Bur1 kinase and its cyclin Bur2 (*S. cerevisiae*) could be recruited to the CTD of RNAPII and stimulate the phosphorylation of Ser2 of CTD at the 5′ end of a gene. In *S. cerevisiae*, the CTK complex consists of a catalytic subunit Ctk1, a cyclin subunit Ctk2 and a co-cyclin factor Ctk3, and is proposed to interact with RNAPII through the Bur1-phosphorylated CTD to phosphorylate Ser2 of CTD at the 3′ end of a gene (Ahn *et al*., 2004; Qiu *et al*., 2009; Bowman and Kelly, 2014; Srivastava and Ahn, 2015). In mammals, the catalytic subunit is CDK12 and the cyclin subunit is CCNK, which is proposed to be the central element in releasing paused RNAPII and moving RNAPII into productive elongation phase (Bowman and Kelly, 2014). Besides, Ctk1 acts as a positive elongation factor, which promotes the transcription elongation. The Ctk1-mediated Ser2 phosphorylation is necessary for efficient recruitment of the histone H3K36 methyltransferase Set2 to the CTD of RNAPII (Xiao *et al*., 2003).

In this study, we characterized the conserved kinase, CTK complex, which phosphorylates Ser2 of RNAPII CTD to regulate *cat-3* expression. In *Neurospora crassa*, CTK complex consists of three subunits: CTK-1 (NCU06685), CTK-2 (NCU04495) and CTK-3 (NCU06470) (Sterner *et al*., 1995; Hautbergue and Goguel, 2001). We revealed that the CTK complex negatively regulates the expression of *cat-3* and *cat-1* genes. Deletion of *ctk-1* resulted in decreased H3K36me3 levels and increased H3ac and H4ac levels at the *cat-3* gene locus, indicating that the CTK complex participates in the transcription elongation of *cat-3*.

Results

CTK mutants are resistant to H_2O_2-induced ROS stress

To identify the factors that contribute to resistance to H_2O_2-induced ROS stress, we performed H_2O_2 sensitivity assays to screen the *Neurospora* knock-out mutants. We found that the *ctk-1*^{KO} strain was resistant to H_2O_2-induced ROS stress in constant light condition on plate (Fig. 1A and B). CTK-1 is the catalytic subunit of the RNA Pol II CTD kinase, which has two other subunits, cyclin CTK-2 and co-cyclin factor CTK-3. Similar results were obtained in the *ctk-2*^{KO} and *ctk-3*^{KO} mutants (Fig. 1A and B). Since CAT-3 is the major catalase in mycelia, these genetic results suggest that the CTK complex may participate in the regulation of *cat-3* expression in *N. crassa*. To test this possibility, we performed western blot assay to analyse the protein levels of CAT-3 in wild-type (WT), *ctk-1*^{KO}, *ctk-2*^{KO} and *ctk-3*^{KO} strains. As shown in Fig. 1C and D, the protein levels of CAT-3 in the *ctk* deletion strains were increased compared with those in the WT strain with or without H_2O_2 treatment. To test whether *ctk* genes deletion affects the activity of CAT-3, we examined the catalase zymograms in the WT and *ctk* deletion strains treated with or without H_2O_2 by an in-gel assay. Proteins separated by PAGE under non-denatured conditions exhibited different bands of catalase activity. As shown in Fig. 1E, the stained bands corresponding to CAT-3 activity were brighter in the *ctk* mutants compared with those in the WT with or without H_2O_2 treatment. In addition, CAT-1 activities were also increased in these mutants (Fig. 1E), suggesting that the CTK complex can negatively regulate the expression of *cat-1* gene in mycelia. Western blot analysis showed that the protein levels of CAT-1 in *ctk* mutant strains were increased compared with those in the WT with or without H_2O_2 treatment (Fig. 1F). Consistent with the protein and catalase activity results, the mRNA levels of *cat-3* and *cat-1* in the *ctk* mutants were also higher than those in the WT (Fig. 1G and H). These results suggest that the elevated expression of *cat-3* and *cat-1* in the *ctk* mutants

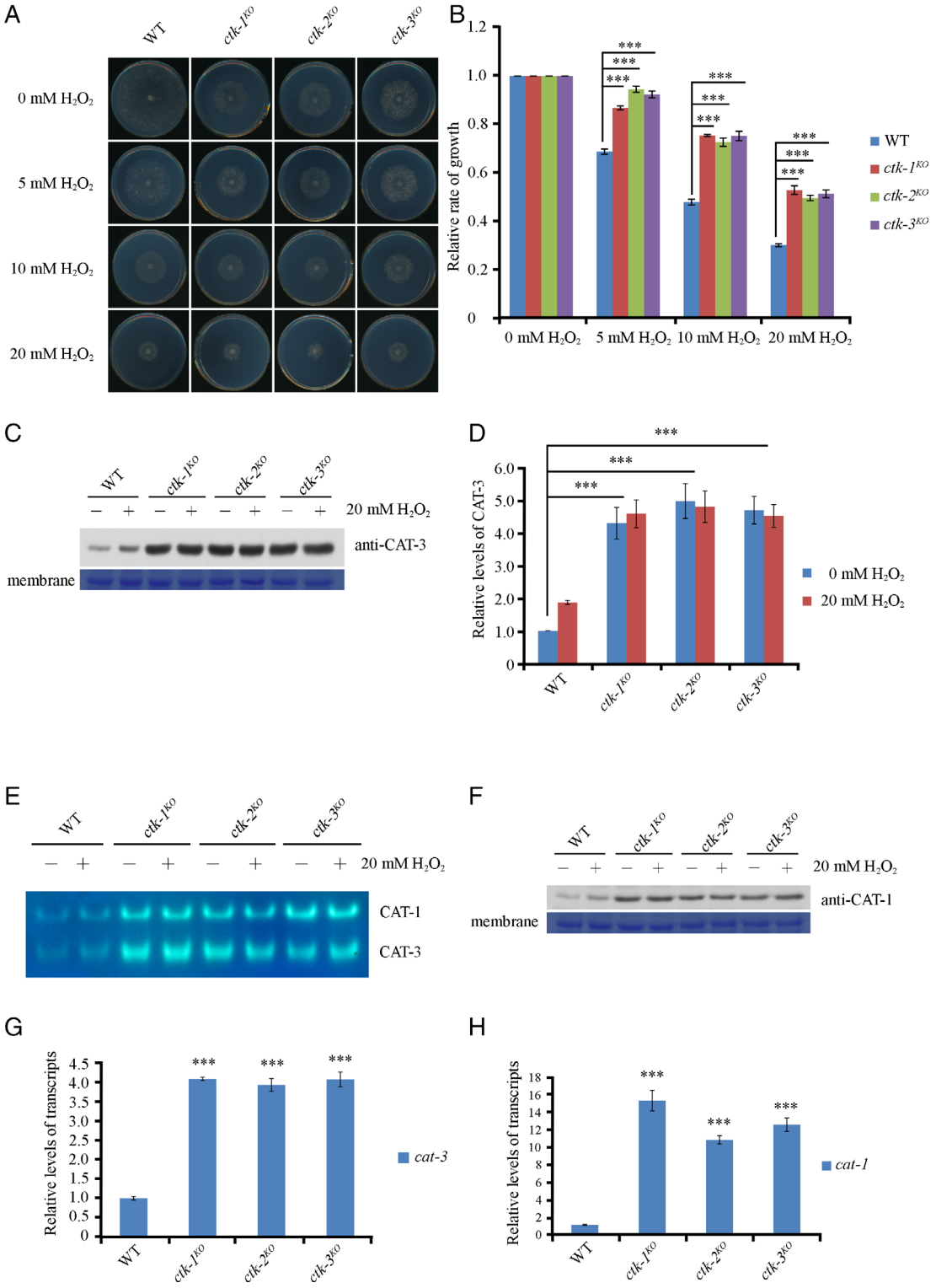


Fig. 1. The CTK complex subunits mutants are resistant to H_2O_2-induced oxidative stress and have high levels of catalases.
A. Relative values of mycelial growth rate with 0, 5, 10 or 20 mM H_2O_2 as indicated. Culturing was performed in 9 cm diameter petri dishes at 25°C under constant light. The relative rate of growth was determined.
B. Relative values were calculated using the values obtained for controls of WT, *ctk-1*^{KO}, *ctk-2*^{KO} and *ctk-3*^{KO} mutants in (A).
C. Western blot analysis showing the levels of CAT-3 protein in the WT, *ctk-1*^{KO}, *ctk-2*^{KO} and *ctk-3*^{KO} mutants with or without H_2O_2. The membranes stained by Coomassie blue represent the total protein in each sample and act as loading control for western blot.
D. Quantification of CAT-3 protein expression levels in (C).
E. Catalase activity assay. Crude extracts from the WT, *ctk-1*^{KO}, *ctk-2*^{KO} and *ctk-3*^{KO} mutants with or without H_2O_2 were subjected to Native-PAGE, and the catalase activity was determined by the in-gel assay.
F. Western blot analysis showing the levels of CAT-1 protein in the WT, *ctk-1*^{KO}, *ctk-2*^{KO} and *ctk-3*^{KO} mutants with or without H_2O_2. The membranes stained by Coomassie blue represent the total protein in each sample and act as loading control for western blot.
G. RT-qPCR analysis showing the levels of *cat-3* mRNA in the WT, *ctk-1*^{KO}, *ctk-2*^{KO} and *ctk-3*^{KO} mutants without H_2O_2.
H. RT-qPCR analysis showing the levels of *cat-1* mRNA in the WT, *ctk-1*^{KO}, *ctk-2*^{KO} and *ctk-3*^{KO} mutants without H_2O_2.
Error bars indicate SD (n = 3). *p < 0.05; **p < 0.01; ***p < 0.001. Student's t test was used.

are responsible for their resistance to H_2O_2-induced ROS stress.

CTK complex negatively regulates the expression of cat-3 *and* cat-1 *genes*

To further confirm the effect of CTK on the catalase levels, three constructs carrying the sequences encoding Myc-tagged CTK-1, CTK-2 or CTK-3 driven by the quinic acid (QA)-inducible promoter were transformed into each corresponding ctk^{KO} strain. The expression of Myc-tagged CTK-1, CTK-2 or CTK-3 induced by QA restored the sensitivity to H_2O_2 of each CTK mutant to those of WT on plate assays (Fig. 2A and B), indicating that the observed phenotype of each mutant was due to the deletion of CTK complex subunits. The leaky expression of Myc-tagged-CTK-1 could be detected in the medium without QA in the complementary strains (Fig. 2C). In 2% glucose medium without QA, ectopic expression of Myc-tagged CTK-1, CTK-2 or CTK-3 in *ctk* mutants restored the activities of CAT-3 to levels similar to those of WT strains (Fig. 2D). Additionally, the intensity of the bands corresponding to CAT-1 activity was also reduced in the transformants (Fig. 2D). Ectopic expression of each CTK subunit efficiently suppressed expression of CAT-3 and CAT-1 in each *ctk* mutants to WT levels (Fig. 2E and F).

Next, we examined whether CTK complex suppresses *cat-3* expression in a dose-dependent manner. Plate assays were performed to examine the growth rates of CTK overexpression strains in medium containing different concentrations of QA following 10 mM H_2O_2 treatment. As shown in Fig. 3A and B, the growth rates of CTK-1 overexpression strain on plates with H_2O_2 decreased significantly, whereas the changes were modest in WT strains, *wt*, pqa-Myc-CTK-2 strain and *wt*, pqa-Myc-CTK-3 strain. The leaky expression of Myc-tagged-CTK-1 in the overexpressing strain could also be detected in the medium without QA (Fig. 3C). Western blot analysis revealed that the CAT-3 protein levels were decreased in *wt*, pqa-Myc-CTK-1 strain compared with that of the WT strains (Fig. 3D). Similarly, levels of CAT-3 activity and *cat-3* mRNA were also reduced in *wt*, pqa-Myc-CTK-1 transformants compared with those in WT strains (Fig. 3E and F). We noticed that the growth rate and CAT-3 protein levels of the mutant strains did not change gradually with increasing QA concentration. This may be due to the QA acting as carbon resource and influence growth rate, somehow causing stress or stimulate the expression of CAT-3. Hence QA concentration increments may introduce a combined effect to the growth of the overexpression strain. Anyways, CAT-3 level was reduced in *wt*, pqa-Myc-CTK-1 strain compared with WT strains in the presence of same concentration of QA, suggesting that the catalytic subunit plays a key role in the regulation of CAT-3 transcription. Taken together, these results indicate that CTK complex negatively regulates *cat-3* gene expression.

The kinase activity of CTK-1 is required for the regulation of the cat-3 *and* cat-1

CTK complex and its catalytic activity is conserved among species. Previous study shows that CTK-1 contributes to the Ser2 phosphorylation of RNAPII CTD in yeast (Bowman and Kelly, 2014). T338 residue of Ctk1 is the phosphorylation site by Cak1 (Cdk-activating kinases 1), which stabilizes cyclin binding and is essential for kinase activation in yeast (Ostapenko and Solomon, 2005). T338A mutation has been shown to cause reduced Ctk1 kinase activity in yeast (Ross *et al*., 2000). In addition, a mutation of the Ctk1catalytic site (D324N) abolishes all kinase activities of Ctk1 in yeast (Ostapenko and Solomon, 2005). When the protein sequence of *Neurospora* CTK-1 was used in a BLAST search against protein databases, its homologues were found to be highly conserved in *Homo sapiens* CDK12, *Saccharomyces cerevisiae* Ctk1 and *Drosophila melanogaster* CDK12 (Fig. 4A). The protein sequence of *Neurospora* CTK-1 displayed high similarity with CDK12 protein of human. To determine whether the kinase activity or structural integrity of CTK-1 protein is required for the suppression of *cat-3* and *cat-1*, we generated a series of CTK-1 mutants by introducing T942A (T338A in yeast), D926N (D324N in yeast) or CDK12Δ (from G787 to H1068) substitution to the qa-Myc-CTK-1 construct. Plate assays showed that the expression of Myc-CTK-1 or Myc-CTK-1^{T942A}, but not Myc-CTK-1^{D926N} or Myc-CTK-$1^{CDK12\Delta}$ rescued the growth defective and the H_2O_2 resistant phenotype of $ctk\text{-}1^{KO}$ strain (Fig. 4B and C), suggesting that the kinase activity of CTK-1 plays an important role in the regulation of ROS resistance. Consistent with the genetic phenotypes of transformants on the plates, the in-gel assays showed that the expression of Myc-CTK-1 and Myc-CTK-1^{T942A} but not Myc-CTK-1^{D926N} or Myc-CTK-$1^{CDK12\Delta}$ significantly suppressed the activities and protein levels of CAT-3 and CAT-1 of $ctk\text{-}1^{KO}$ strain (Fig. 4D-G). Furthermore, RT-qPCR results revealed that the elevated levels of *cat-3* and *cat-1* mRNA in $ctk\text{-}1^{KO}$ strain were suppressed by Myc-CTK-1 and Myc-CTK-1^{T942A} but not Myc-CTK-1^{D926N} or Myc-CTK-$1^{CDK12\Delta}$ proteins (Fig. 4H and I). Taken together, these data demonstrated that the catalytic activity of CTK-1 protein was required for its role in the regulation of *catalase* expression.

CTK-1 phosphorylates Ser2 residues of RNAPII CTD at cat-3 *locus*

Previous studies showed that the CTK complex is responsible for Ser2 phosphorylation of RNAPII CTD

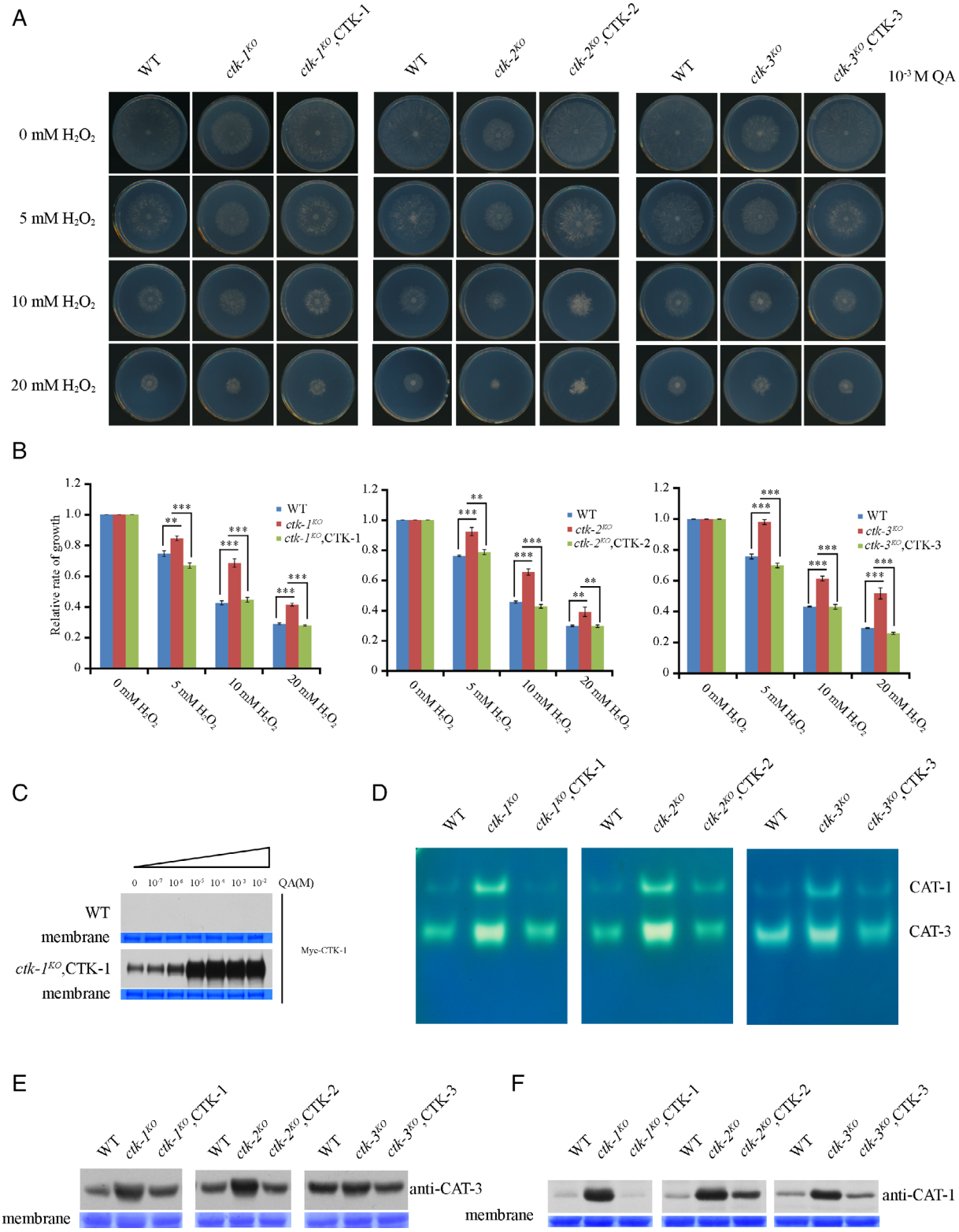


Fig. 2. Ectopic expression of Myc-tagged CTK-1, CTK-2 or CTK-3 in CTK mutants can rescue the resistant phenotype.
A. Growth of WT, *ctk-1*^{KO}, *ctk-2*^{KO} or *ctk-3*^{KO} and CTK-1, CTK-2 or CTK-3 transformants in plates under the treatments of 0, 5, 10 or 20 mM H_2O_2. Quinic acid (QA) was used to induce the *qa-2* promoter.
B. Relative values were calculated using the values obtained for controls of WT, *ctk-1*^{KO}, *ctk-2*^{KO} or *ctk-3*^{KO} and CTK-1, CTK-2 or CTK-3 transformants in (A).
C. The expression of Myc-tagged CTK-1 in the WT and *ctk-1*^{KO}, CTK-1. Different concentrations of QA were used to induce the *qa-2* promoter.
D. Catalase activity assay. Crude extracts from the WT, *ctk-1*^{KO}, *ctk-2*^{KO} or *ctk-3*^{KO} and CTK-1, CTK-2 or CTK-3 transformants were subjected to Native-PAGE and catalase activities were determined by the in-gel assay.
E. Western blot analysis showing the levels of CAT-3 protein in the WT, *ctk-1*^{KO}, *ctk-2*^{KO} or *ctk-3*^{KO} and CTK-1, CTK-2 or CTK-3 transformants. The membranes stained by Coomassie blue represent the total protein in each sample and act as loading control for western blot.
F. Western blot analysis showing the levels of CAT-1 protein in the WT, *ctk-1*^{KO}, *ctk-2*^{KO} or *ctk-3*^{KO} and CTK-1, CTK-2 or CTK-3 transformants. The membranes stained by Coomassie blue represent the total protein in each sample and act as loading control for western blot.

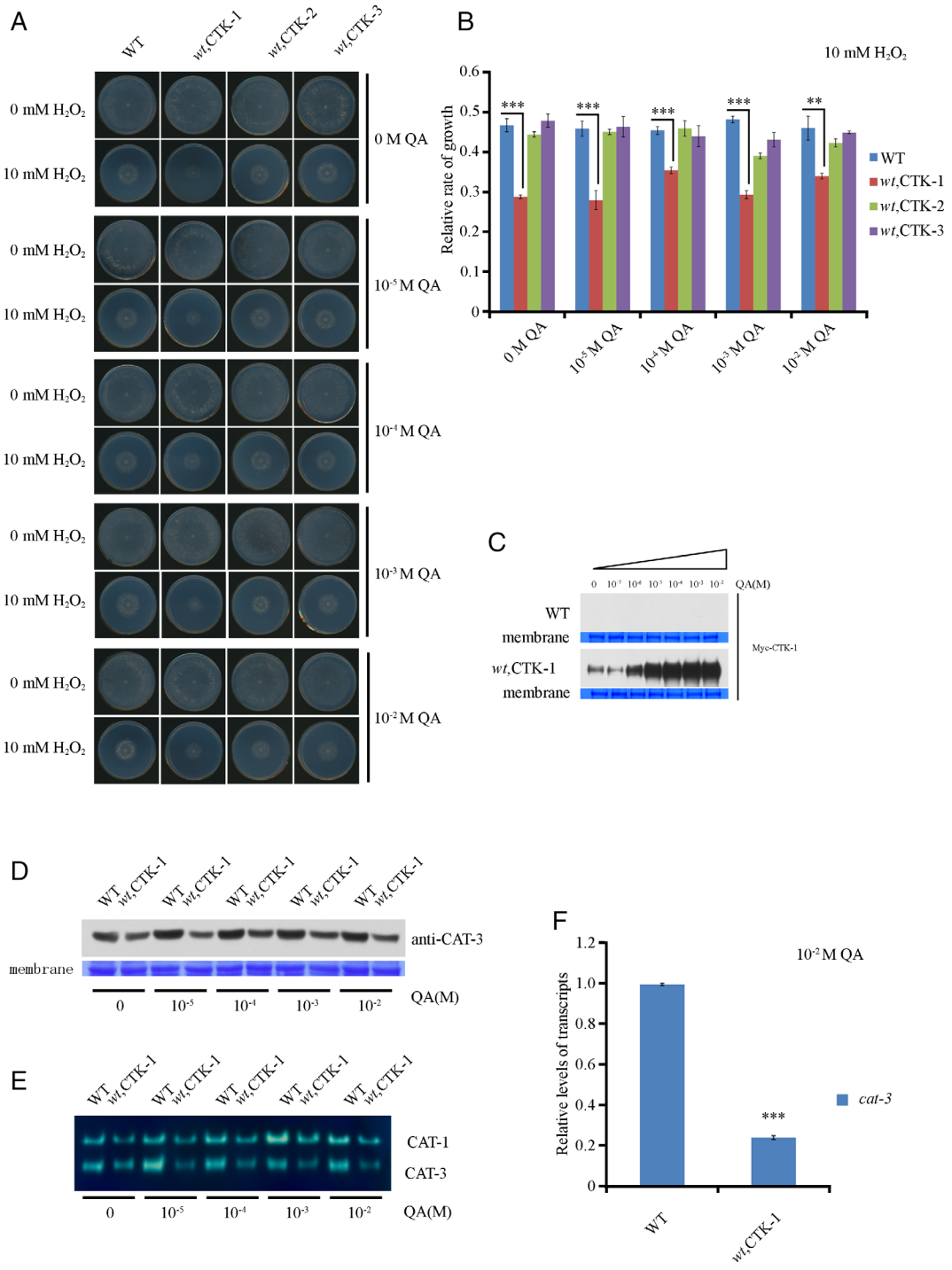


Fig. 3. The CTK-1 overexpressing strains exhibit decreased *cat-3* expression and are sensitive to H_2O_2.
A. Growth of WT and CTK overexpressing strains in plates under the treatment of 10 mM H_2O_2. Culturing was performed in petri dishes at 25°C under constant light. Different concentrations of QA were used to induce the *qa-2* promoter.
B. Relative values were calculated using the values obtained for controls of WT and CTK overexpressing strains in (A).
C. The expression of Myc-tagged CTK-1 in the WT and *wt*, CTK-1. Different concentrations of QA were used to induce the *qa-2* promoter.
D. Western blot analysis showing the levels of CAT-3 protein in the WT and *wt*, CTK-1 strain under the different concentrations of QA. The membranes stained by Coomassie blue represent the total protein in each sample and act as loading control for western blot.
E. Catalase activity assay. Crude extracts from the WT, *wt* and CTK-1 strain were subjected to Native-PAGE and the catalase activities were determined by the in-gel assay. Different concentrations of QA were used to induce the *qa-2* promoter.
F. RT-qPCR analyses showing the levels of *cat-3* mRNA in the WT and *wt*, CTK-1 strain. 10^{-2} M QA was used to induce the *qa-2* promoter.
Error bars indicate SD (n = 3). *p < 0.05; **p < 0.01; ***p < 0.001. Student's t test was used.

(Sterner *et al*., 1995; Hautbergue and Goguel, 2001; Bowman and Kelly, 2014). To investigate the role of CTK-1 on *cat-3* transcription, we measured the phosphorylation levels of Ser2 on RNAPII CTD using the specific antibody for CTD Ser2p in the WT, *ctk-1*^{KO} and different *ctk-1*^{KO} transformant strains. The levels of CTD

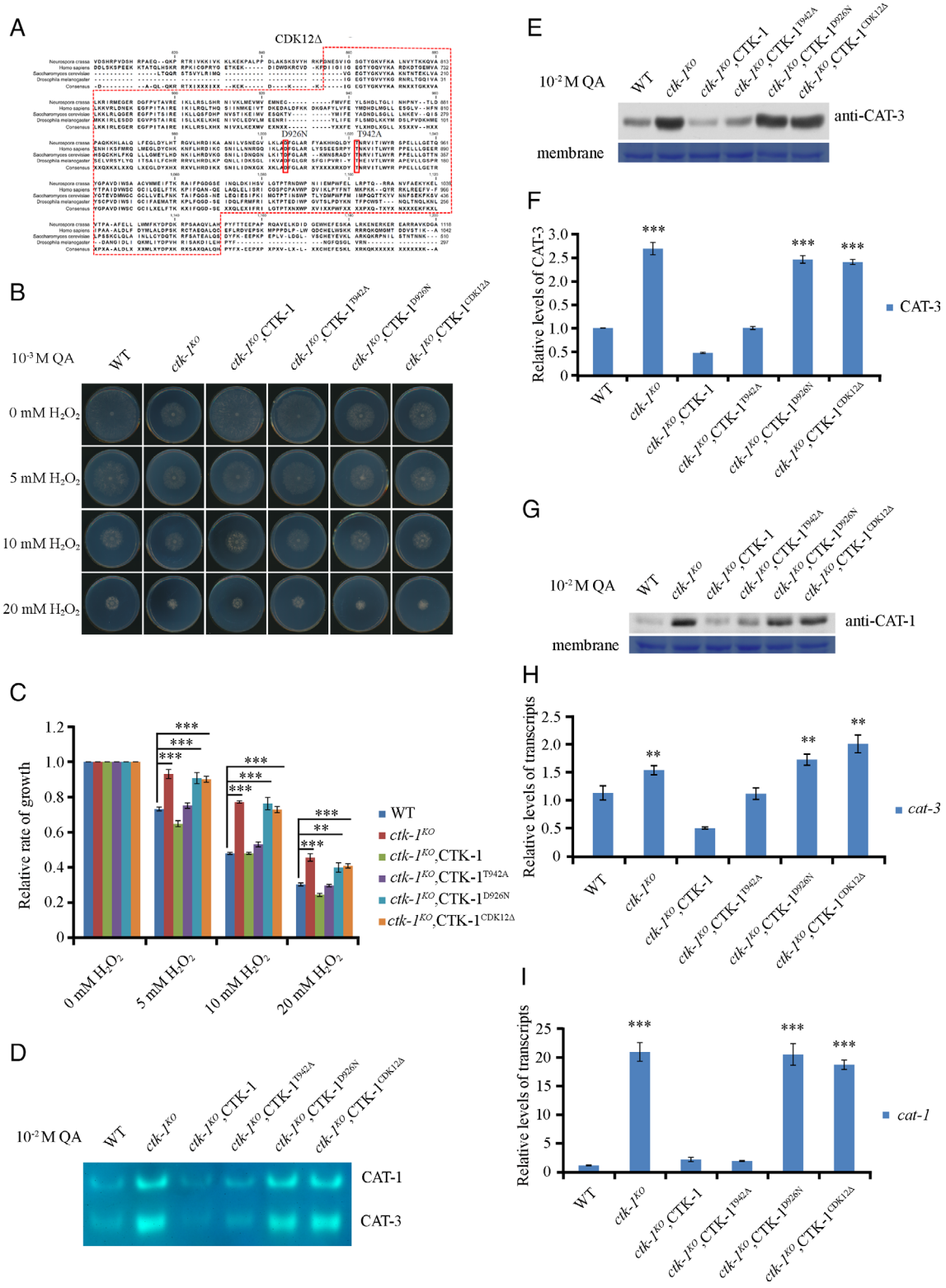


Fig. 4. Legend on next page.

Ser2 phosphorylation were very low in the *ctk-1*^{KO} strain compared with that in WT strains (Fig. 5A). However, the expression of Myc-CTK-1 and Myc-CTK-1^{T942A} but not Myc-CTK-1^{D926N} or Myc-CTK-1$^{CDK12\Delta}$ restored the Ser2 phosphorylation of RNAPII CTD (Fig. 5A), indicating that the CTK-1 is the major kinase for CTD Ser2 residues in *Neurospora*, and the Myc-CTK-1^{D926N} and Myc-CTK-1$^{CDK12\Delta}$ are catalytic dead proteins. To assess the role of CTK-1-mediated Ser2 phosphorylation of RNAPII in the regulation of *cat-3* gene, we performed ChIP assay using the CTD Ser2p specific antibody to check the recruitment of RNAPII Ser2p at *cat-3* locus in WT and *ctk-1*^{KO} strain. The oligo nucleotide primer pairs were designed to target an approximately 8.5-kb region from the proximal 6-kb upstream region of *cat-3* transcription start site (TSS) to the *cat-3* ORF 3′ region in the WT strains (primer pairs 1–8; Fig. 5B). ChIP-qPCR results showed that during *cat-3* gene transcription, the total level of RNAPII CTD Ser2 phosphorylation was increased at the *cat-3* locus (primer pairs 6–8) in WT strains (Fig. 5C), whereas the total level of Ser2 phosphorylation remains low in *ctk-1*^{KO} strain (Fig. 5C), confirming that the Ser2 phosphorylation of RNAPII CTD was also abolished at *cat-3* locus in *ctk-1*^{KO} strain. To determine that the high expression of *cat-3* is due to the increased recruitment of RNAPII at *cat-3* locus in *ctk-1*^{KO} strain, we carried out ChIP assays using available commercial CTD specific antibody in WT and *ctk-1*^{KO} strains. Consistent with high expression of *cat-3* in *ctk-1*^{KO} strains, qPCR results revealed that the binding of RNAPII CTD at *cat-3* locus in *ctk-1*^{KO} strain was increased compared with those in WT strains (Fig. 5D). To confirm this possibility, we generated an antiserum against *Neurospora* RPB-1 and repeated ChIP assays using this RPB-1 specific antibody. As shown in Fig. 5E, the recruitment levels of RPB-1 at *cat-3* locus in *ctk-1*^{KO} strain also increased compared with those in the WT strains. These results demonstrated that loss of CTK-1 catalytic function led to increased recruitment of RNAPII at *cat-3* gene and elevated *cat-3* expression.

CTK-1-mediated Ser2 phosphorylation recruits SET-2 to cat-3 *locus which maintains proper acetylation status at the* cat-3 *locus*

During transcription elongation, the phosphorylated RNAPII Ser2 also acts as a platform for recruiting SET-2, a histone methyltransferase for H3K36 trimethylation (Li *et al*., 2002; Krogan *et al*., 2003; Li *et al*., 2003). To test whether CTK-1-mediated Ser2p is required for SET-2 recruitment at *cat-3* locus, we performed ChIP assay using a specific anti-SET-2 antibody. As shown in Fig. 6A, the SET-2 enrichment was greatly diminished in *ctk-1*^{KO} strain compared with those in the WT strains. Furthermore, ChIP assay using H3K36me3 antibody revealed that the deletion of CTK-1 led to a dramatic decrease of H3K36me3 levels at *cat-3* locus compared with levels in the WT strains (Fig. 6B). These results indicated that CTK-1-mediated Ser2 phosphorylation of RNAPII CTD leads to SET-2 recruitment and H3K36me3 at *cat-3* locus during transcription elongation. The dramatic decrease of SET-2 and H3K36me3 levels at the *cat-3* locus in the *ctk-1*^{KO} strain prompted us to examine whether the SET-2 functions in the regulation of *cat-3* gene expression in *Neurospora*. As shown in Fig. 6C and D, the *set-2*^{KO} strain was resistant to H_2O_2-induced ROS stress in constant light condition on plate compared with those of WT strains. Consistent with the plate assay results, deletion of *set-2* also elevated both CAT-3 protein and activity levels (Fig. 6E–G). Similarly, *cat-3* mRNA levels in *set-2*^{KO} mutants were dramatically increased compared with those in the WT strains (Fig. 6H). These results suggest that CTK-1 regulates *cat-3* transcription, in part, through SET-2-mediated H3K36me3 at the *cat-3* locus.

In *S. cerevisiae*, transcribed genes maintain states of hypoacetylation due to Set2-mediated H3K36me3 (Keogh *et al*., 2005; Li *et al*., 2007; Smolle *et al*., 2012; Venkatesh *et al*., 2012). Our recent study also showed that deletion of SET-2 resulted in histone hyperacetylation and thus loosens chromatin structure at *frq* locus (Sun *et al*., 2016). We performed ChIP assays

FIGURE 4 The kinase activity of CTK-1 contributes to the suppression of *cat-3* expression and the resistance to H_2O_2-induced oxidative stress.
A. Amino acid sequence alignment of the CDK12 domains of CTK-1 from *Neurospora crassa*, *Homo sapiens*, *Saccharomyces cerevisiae* and *Drosophila melanogaster*.
B. Growth of WT, *ctk-1*^{KO} and CTK-1 transformants in plates under the treatment of 0, 5, 10 or 20 mM H_2O_2. 10^{-3} M QA was used to induce the *qa-2* promoter.
C. Relative values were calculated using the values obtained for controls of WT, *ctk-1*^{KO} and CTK-1 transformants in (B).
D. Catalase activity assay. Crude extracts from the WT, *ctk-1*^{KO} and CTK-1 transformants were subjected to Native-PAGE, and the catalase activities were determined by the in-gel assay. 10^{-2} M QA was used to induce the *qa-2* promoter.
E. Western blot analysis showing the levels of CAT-3 protein in the WT, *ctk-1*^{KO} and CTK-1 transformants. The membranes stained by Coomassie blue represent the total protein in each sample and act as loading control for western blot. 10^{-2} M QA was used to induce the *qa-2* promoter.
F. Quantification of CAT-3 protein expression levels in (E).
G. Western blot analysis showing the levels of CAT-1 protein in the WT, *ctk-1*^{KO} and CTK-1 transformants. The membranes stained by Coomassie blue represent the total protein in each sample and act as loading control for western blot. 10^{-2} M QA was used to induce the *qa-2* promoter.
H. RT-qPCR analysis showing the levels of *cat-3* mRNA in the WT, *ctk-1*^{KO} and CTK-1 transformants. 10^{-2} M QA was used to induce the *qa-2* promoter.
I. RT-qPCR analysis showing the levels of *cat-1* mRNA in the WT, *ctk-1*^{KO} and CTK-1 transformants. 10^{-2} M QA was used to induce the *qa-2* promoter.
Error bars indicate SD (n = 3). *p < 0.05; **p < 0.01; ***p < 0.001. Student's t test was used.

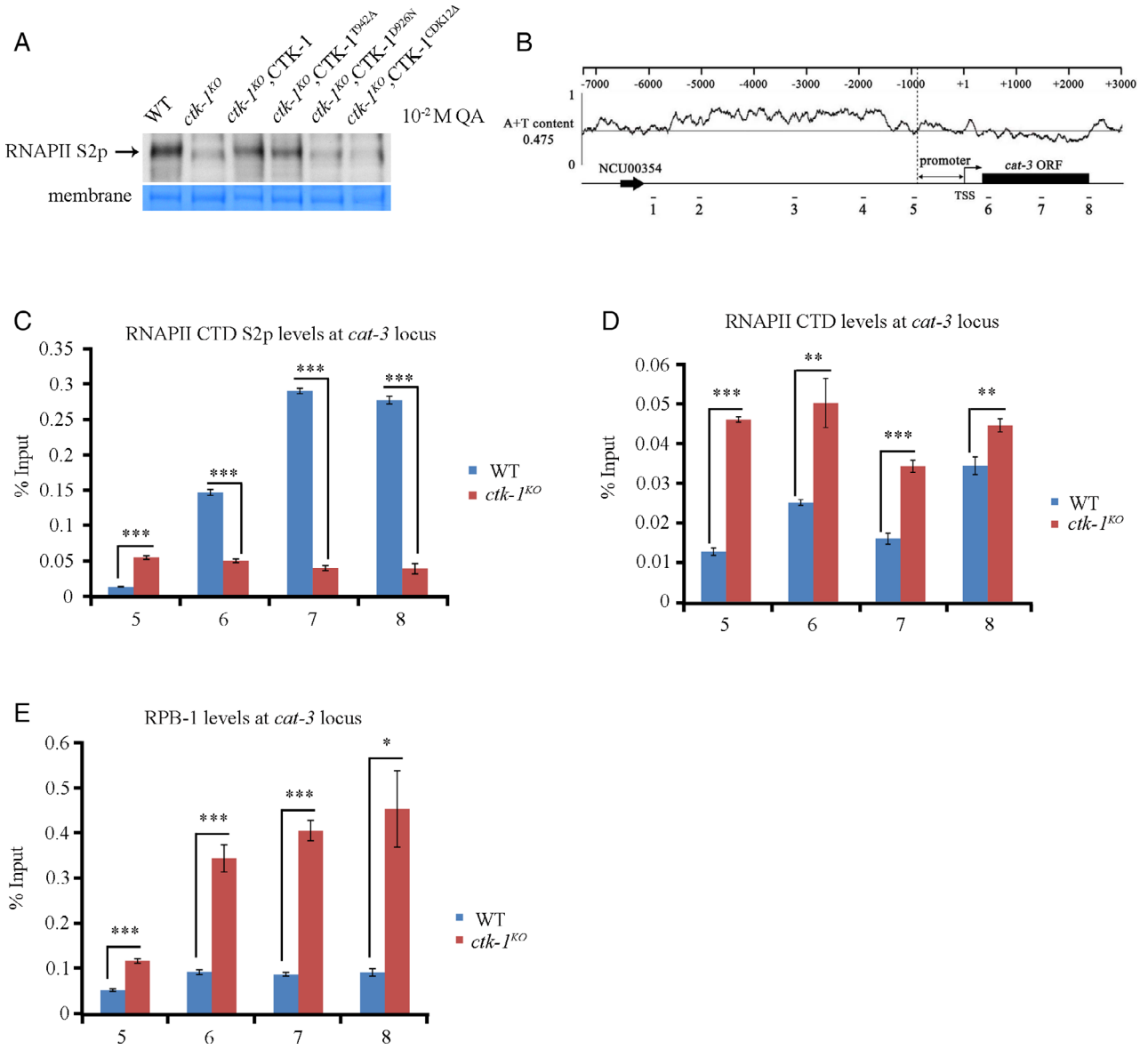


Fig. 5. CTK-1 is required for Ser2 phosphorylation of RNAPII CTD.
A. Western blot analysis showing the levels of RNAPII CTD Ser2p in WT, *ctk-1*^{KO} and CTK-1 transformants. The membranes stained by Coomassie blue represent the total protein in each sample and act as loading control for western blot. 10^{-2} M QA was used to induce the *qa-2* promoter.
B. Schematic depiction of a 5-kb AT-rich DNA region located between *cat-3* (NCU00355) and NCU00354 on linkage group III of *Neurospora* genome. Short black lines (primer pairs 1–8) under the schematic indicate the regions tested by ChIP-qPCR. TSS, transcription start site; ORF, open reading frame.
C. ChIP analysis showing the recruitment of RNAPII CTD Ser2p at different regions of the *cat-3* locus in the WT and *ctk-1*^{KO} strain (primer pairs 5–8).
D. ChIP analysis showing the enrichment of CTD at different regions of the *cat-3* locus in the WT and *ctk-1*^{KO} strain (primer pairs 5–8).
E. ChIP analysis showing the enrichment of RPB-1 at different regions of the *cat-3* locus in the WT and *ctk-1*^{KO} strain (primer pairs 5–8).
Error bars indicate SD (n = 3). *p < 0.05; **p < 0.01; ***p < 0.001. Student's t test was used.

using anti-H3 and H4 acetylation antibody to check the histone acetylation statuses of nucleosomes at *cat-3* gene body in WT and *ctk-1*^{KO} strains. ChIP-qPCR results revealed that the relative levels of H3ac and H4ac increased at the *cat-3* gene body in *ctk-1*^{KO} strain (Fig. 7A and B). Our previous research showed that CPC1 is a key activator for *cat-3* expression via directly binding to the promoter of *cat-3* (Dong *et al*., 2018; Qi *et al*., 2018). To test whether elevated histone acetylation caused the increased binding of CPC1 proteins at *cat-3* locus for its expression in *ctk-1*^{KO} strain, we performed ChIP assay using the specific anti-CPC1 antibody. As

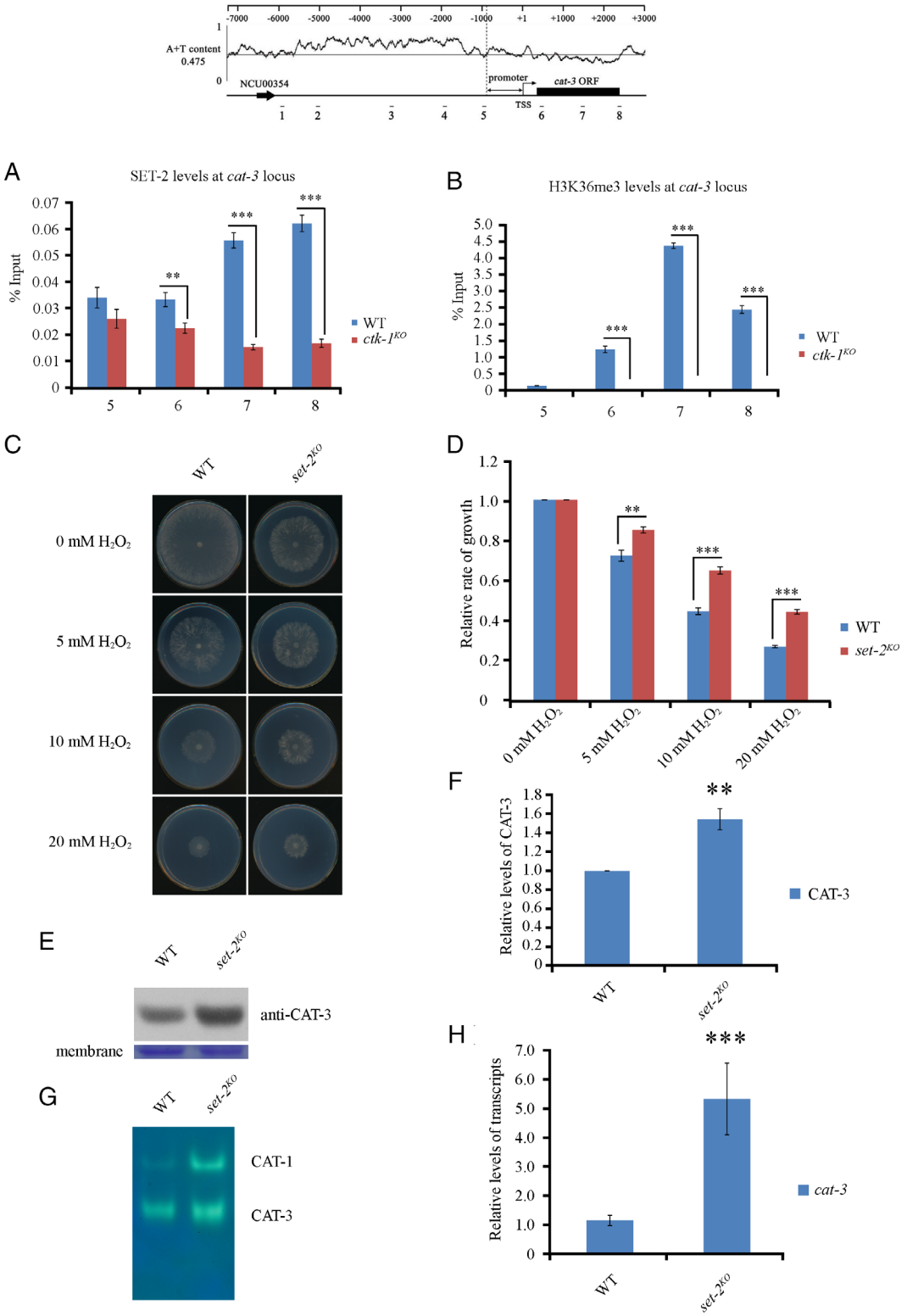
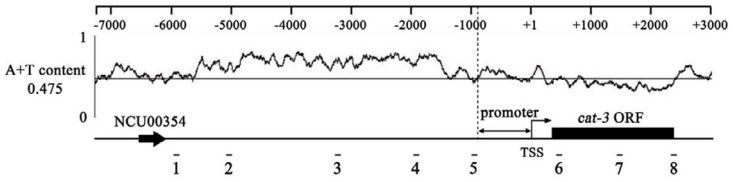


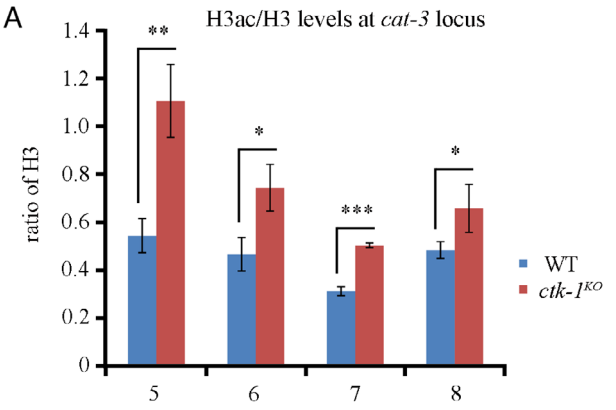
Fig. 6. CTK-1 regulates *cat-3* transcription through SET-2-mediated H3K36me3 at the *cat-3* locus.
A. ChIP analysis showing the enrichment of SET-2 at different regions of the *cat-3* locus in the WT and *ctk-1*^{KO} strain (primer pairs 5–8).
B. ChIP analysis showing the enrichment of H3K36me3 at different regions of the *cat-3* locus in the WT and *ctk-1*^{KO} strain (primer pairs 5–8).
C. Growth of WT, *set-2*^{KO}strains in plates under the treatments of 0, 5, 10 or 20 mM H_2O_2.
D. Relative values were calculated using the values obtained for controls of WT, *set-2*^{KO} mutants in (C).
E. Western blot analysis showing the levels of CAT-3 protein in the WT, *set-2*^{KO} mutants. The membranes stained by Coomassie blue represent the total protein in each sample and act as loading control for western blot.
F. Quantification of CAT-3 protein expression levels in (E).
G. Catalase activity assay. Crude extracts from the WT, *set-2*^{KO} mutants were subjected to Native-PAGE and the catalase activities were determined by the in-gel assay.
H. RT-qPCR analyses showing the levels of *cat-3* mRNA in the WT and *set-2*^{KO} mutants.
Error bars indicate SD (n = 3). *p < 0.05; **p < 0.01; ***p < 0.001. Student's t test was used.

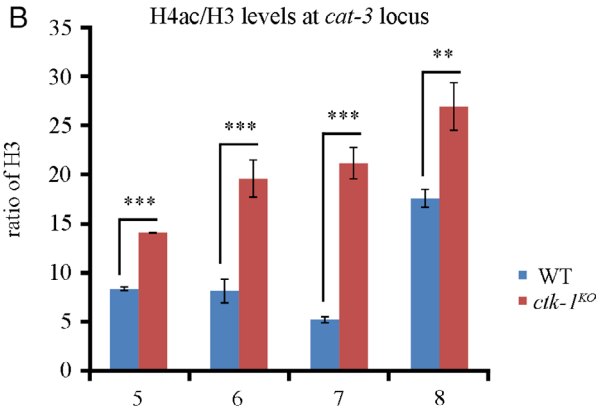
shown in Fig. 7C, the enrichment of CPC1 was increased at the promoter and ORF of the *cat-3* locus (primer pairs 5–8) in *ctk-1*^{KO} strain compared with those in WT strain. Interestingly, RT-qPCR analysis revealed that the transcription levels of *cpc-1* elevated in *ctk-1*^{KO} strain compared with those in the WT strain (Fig. 7D), implying that the enhanced CPC1 expression results in its elevated recruitment at *cat-3* locus. Taken together, these results indicated that deletion of CTK-1 led to decreased H3K36me3 levels and loosen chromatin structure at the *cat-3* gene body by histone hyperacetylation, which facilitated the access of transcriptional machinery and promotes *cat-3* gene expression.

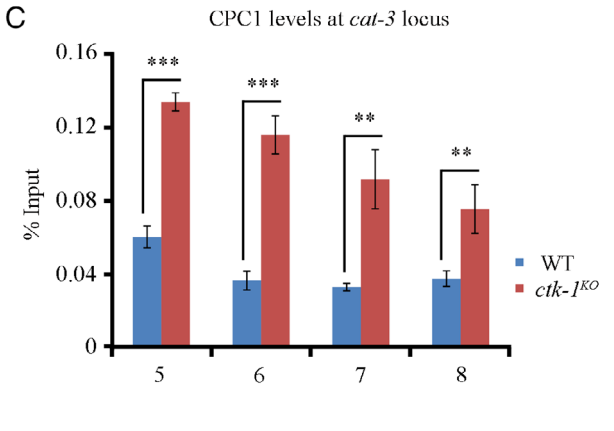
Discussion

Transcriptional regulation of inducible gene elements is an important step in responding to environmental or









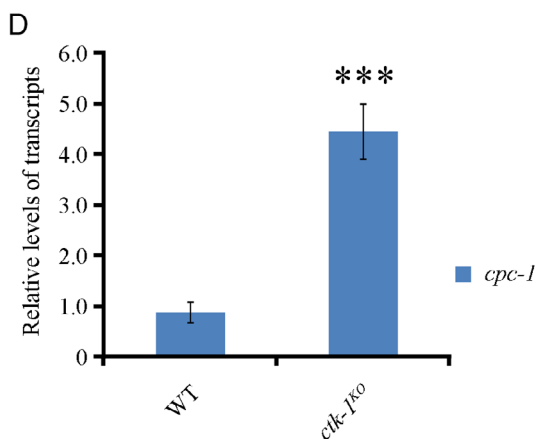


Fig. 7. CTK-1-mediated Ser2 phosphorylation affects proper histone acetylation status at the *cat-3* locus and *cpc-1* expression.
A. ChIP analysis showing the acetylation of H3 at *cat-3* locus in the WT and *ctk-1*^{KO} strain (primer pairs 5–8).
B. ChIP analysis showing the acetylation of H4 at *cat-3* locus in the WT and *ctk-1*^{KO} strain (primer pairs 5–8).
C. ChIP analysis showing the enrichment of CPC1 at different regions of the *cat-3* locus in the WT and *ctk-1*^{KO} strain (primer pairs 5–8).
D. RT-qPCR analyses showing the levels of *cpc-1* mRNA in the WT and *ctk-1*^{KO} mutants.
Error bars indicate SD (n = 3). *p < 0.05; **p < 0.01; ***p < 0.001. Student's t test was used.

developmental signals. Our previous studies have demonstrated that a bZIP transcription factor, CPC1/GCN4 is responsible for activating *catalase-3* transcription (Dong *et al*., 2018; Qi *et al*., 2018). In this study, we found that the transcriptional regulator complex CTK, which is responsible for the phosphorylation of the Ser2 residues of RNAPII CTD, is also involved in the regulation of *cat-3* transcription in *N*. *crassa*. Deletion of each subunit gene of CTK complex in *N*. *crassa* genome leads to robust CAT-3 and CAT-1 activation and high resistance to H_2O_2 treatment. CTK-1 is the catalytic subunit of CTK complex, and overexpression of CTK-1 results in higher sensitivity to H_2O_2 and decreased levels of CAT-3 protein and *cat-3* mRNA. Furthermore, elevated expression of *cat-3* and *cat-1* is dependent on the kinase activity of CTK-1. Biochemical data demonstrated that *N*. *crassa* CTK-1 is responsible for the phosphorylation of Ser2 residues of RNAPII CTD at *cat-3* locus. According to previous studies and our experiments, we assume that mutation of CTK-1 alters chromatin accessibility and increases *cat-3* expression. Additionally, CAT-3 induction is impeded in ctk^{KO} strains treated with H_2O_2, indicating that functional CTK complex serves as a regulatory target for environmental or developmental stimuli. Taken together, our results demonstrated that CTK complex is critical for proper *cat-3* and *cat-1* transcription.

CTK-1 negatively regulates *cat-3* transcription, and the role of CTK-1 on gene expression has been reported in previous studies. To identify the underlying molecular mechanism, we performed a series of experiments and found that Ser2 phosphorylation of RNAPII CTD at *cat-3* locus in $ctk\text{-}1^{KO}$ strain was extremely low (Fig. 5C). Several studies in multiple systems support that the histone methyltransferase Set2, which methylates histone H3K36, is associated with CTD phosphorylation. In this study, we found that deletion of CTK-1 led to a dramatic decrease of SET-2 and H3K36me3 levels at the *cat-3* locus (Fig. 6A and B). These results suggest that the Ctk1-mediated Ser2 phosphorylation is necessary for efficient recruitment of the histone H3K36 methyltransferase Set2 to the CTD of RNAPII.

Nucleosomes generally act as barriers to transcription, by hampering RNAPII recruitment, movement and related transcription machineries. Histone modifications directly affect the chromatin structures, which play important roles in regulating gene transcription. Loss of H3K36me3 results in increased histone acetylation at the open reading frame (ORF) of genes and leads to cryptic transcription due to promoters within ORFs. Previous research shows that *cat-3* gene responds to H_2O_2 stress; its expression level and H3 acetylation at its locus are significantly increased after H_2O_2 treatment. To further test how the chromatin structure is loosened in ctk^{KO} strains, we performed ChIP assay and found that the levels of H3 and H4 acetylation increases in ctk^{KO} strains (Fig. 7A and B), which facilitates the access of CPC1 at *cat-3* gene locus (Fig. 7C). Our finding suggests that CTK-1 complex regulates the transcription elongation of *cat-3* gene and the histone hyperacetylation affects gene expression efficiency in a context-dependent manner.

In yeast, the role of phosphorylating CTD Ser2 is divided between Ctk1 and Bur1. In *N*. *crassa*, the CTK-1 is the major kinase for CTD Ser2 residues. We tried to knockout *bur-1* gene, but failed to get a homozygous *bur-1* knockout strain, suggesting that although BUR-1 is not the main CTD Ser2 phosphorylation kinase in *Neurospora*, it is required for cell survival. It is worth noting that BUR-1 and CTK-1 are unable to functionally substitute even when overexpressed in yeast, suggesting that they have distinct functions (Keogh *et al*., 2003).

Many studies in yeast and humans demonstrate the role of H3K36 methylation in transcriptional activation. However, H3K36 methylation has also been implicated in transcriptional repression, alternative splicing, dosage compensation, DNA replication, DNA repair and DNA methylation. In higher eukaryotes, the abnormal levels of H3K36 methylation cause developmental defects and diseases (Wagner and Carpenter, 2012). In this study, we demonstrate that the CTK-1 play essential roles in the phosphorylation of CTD Ser2, which repress the expression of *cat-3* through the SET-2 and H3K36me3 pathway. We also find that *cat-1* is negatively regulated by the CTK complex. As the *catalase* and the ROS clearance system are highly conserved from prokaryotes to eukaryotes, it appears to be important to unravel the oxidant stress adaption pathways to better understand and implement relevant measures for the proper treatment of stress and disease.

Materials and methods

Strains and culture conditions

The 87–3 (*bd*, *a*) strain was used as the wild-type strain in this study (Belden *et al*., 2007). The $ku70^{RIP}$ (*bd*, *a*) strain, generated previously (He *et al*., 2006), was used as the host strain for creating the *ctk-1* (NCU06685), *ctk-2* (NCU04495) or *ctk-3* (NCU06470) knock-out mutant by deleting the entire ORF through homologous recombination using a protocol described previously (Colot *et al*., 2006). The $set\text{-}2^{KO}$ strain was generated previously (Zhou *et al*., 2013; Sun *et al*., 2016).The plasmid containing *qa-2* promoter driven the CTK-1 ORF and its 3′-UTR (pqa-5Myc-6His-CTK-1) was used as the template for mutagenesis, and three mutations of CTK-1 ($CTK\text{-}1^{T942A}$, $CTK\text{-}1^{D926N}$ or $CTK\text{-}1^{CDK12\Delta}$) were generated. Afterwards, the mutated plasmids (pqa-5Myc-6His-$CTK\text{-}1^{T942A}$, pqa-5Myc-6His-$CTK\text{-}1^{D926N}$ or pqa-5Myc-6His-$CTK\text{-}1^{CDK12\Delta}$) were transformed into $ctk\text{-}1^{KO}$ (*bd*, $his\text{-}3^{-}$)

strains to get *ctk-1*^{KO}, Myc-CTK-1^{T942A}, *ctk-1*^{KO}, Myc-CTK-1^{D926N} and *ctk-1*^{KO}, Myc-CTK-1$^{CDK12\Delta}$ transformants. The *wt*, pqa-Myc-CTK-1 were created by transferring pqa-5Myc-6His-CTK-1 constructs into the *his-3* locus of 301–6 (*bd*, *his-3*$^{-}$) host strain.

Liquid cultures were grown in minimal medium (1× Vogel's, 2% glucose) with different concentration of H_2O_2. When QA was used, liquid cultures were grown in low-glucose medium (1× Vogel's, 0.1% glucose, 0.17% arginine). Liquid cultures were grown at 25°C with shaking for 18 h in constant light (LL).

For protein and RNA analysis, the 5- or 7-day-old conidia were inoculated in petri dishes with 50 ml liquid medium containing 1× Vogel's and 2% glucose under static culture condition at 25°C for 1–2 days. The mycelium mat (adhered hyphae) was cut into disks for quantification. For each strain, equal amounts of mycelium disks were transferred into flasks with 50 ml fresh liquid medium and then incubated with agitation at 130 rpm for 18 h in constant light (LL) at 25°C. All cell extracts from the adhered mycelium were used for performing protein and RNA analysis.

Protein analysis

Protein extraction, quantification and western blot analysis were performed as described previously (Yang *et al*., 2014). Equal amounts of total protein (40 μg) were loaded into each lane. After electrophoresis, proteins were transferred onto PVDF membrane. Western blot analysis was performed using antibodies against the proteins of interest. Densitometric analysis from three independent experiments was calculated with Quantity One® 1-D analysis software made by Bio-Rad Laboratories.

RNA analysis

For quantitative real-time reverse transcriptase-PCR, total RNA was isolated with Trizol agent and treated with DNase I to remove genomic DNA according to the manufacturer's protocol. Each RNA sample (total RNA, 5 μg) was subjected to reverse transcription with M-MLV reverse transcriptase (Promega) and then amplified by real-time PCR. The primers used for qPCR were shown in Table S1. The relative values of gene expression were calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) by comparing the cycle number for each sample with that for the untreated control. The results were normalized to the expression levels of β-tubulin gene.

In-gel assay for catalase

Cell extracts of mycelium disks cultured 18 h in liquid medium were used for the zymogram. Grinded tissues were mixed with ice-cold extraction buffer containing 50 mM HEPES (pH 7.4), 137 mM NaCl, 10% glycerol and protease inhibitors, Pepstatin A (1 μg/ml), Leupeptin (1 μg/ml), PMSF (1 mM) and centrifuged at 10,000*g* for 10 min at 4°C. The protein concentration was measured by Bio-Rad protein assay dye at 595 nm.

For the in-gel assay, catalase activity was determined as described previously (Lledías *et al*., 1998). Equal amounts of total protein (40 μg) were loaded into a 7.5% native polyacrylamide slab gel. After electrophoresis, the gel was immersed in 10 mM H_2O_2 with shaking for 10 min and then in a 1:1 mixture of freshly prepared 1% potassium hexacyanoferrate (III) and 1% iron (III) chloride hexahydrate. Catalase activity was visualized as a band where H_2O_2 was decomposed by catalase.

Plate assay

The medium for plate assays contained 1× Vogel's salts, 0.1% glucose, 0.17% arginine, 50 ng/ml biotin and 1.5% agar with different concentration of H_2O_2. In the plate medium containing QA, 0.1% glucose was replaced with the 10^{-3} M QA.

The 5- or 7-day-old conidia were inoculated in petri dishes with 50 ml liquid medium containing 1× Vogel's and 2% glucose under static culture condition at 25°C for 1–2 days. The disks of mycelium mat (adhered hyphae) were cut with a cork borer for quantification. A piece of mycelium mat of WT or mutant strain was inoculated at the centre of the petri dishes and was grown under constant light (LL) with medium containing 0, 5, 10 or 20 mM H_2O_2. When the WT strain grew and covered the medium without H_2O_2, all plates were scanned and the average growth rate of each strain relative to that in medium without H_2O_2 (0 mM) was calculated. Each experiment was performed at least three times independently.

Generation of antiserum against RPB-1

GST-RPB1 (containing RPB-1 amino acids D804-T1098) fusion proteins were expressed in BL21 cells, and soluble recombinant proteins were purified and used as the antigens to generate rabbit polyclonal antiserum, as described previously (Xu *et al*., 2010; Zhao *et al*., 2010).

ChIP analysis

Chromatin immunoprecipitation (ChIP) assays were performed as described previously (Zhou *et al*., 2013). Briefly, *N. crassa* tissues were fixed with 1% formaldehyde for 15 min at 25°C with shaking. Glycine was added at a final concentration of 125 mM, and samples were incubated for another 5 min. The cross-linked tissues were grinded and re-suspended at 0.5 g in 6 ml lysis buffer containing protease inhibitors (1 mM PMSF,

1 μg/ml leupeptin and 1 μg/ml pepstatin A). Chromatin was sheared by sonication to approximately 500–1000 bp fragments. A 1 ml aliquot of protein solution (2 mg/ml) was used for each immunoprecipitation reaction, and 10 μl was kept as the input DNA. The ChIP was carried out with 10 μl of anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [H5] (ab24758; Abcam), 10 μl of anti-RNA polymerase II CTD repeat YSPTSPS antibody [8WG16] (ab817; Abcam), 3 μl of anti-H3K36me3 antibody (ab9050; Abcam), 3 μl of anti-H3 antibody (2650; CST), 3 μl of anti-H3ac antibody (07–473; Millipore), 10 μl of anti- SET-2 antibody, 10 μl of anti-RPB-1 antibody and 10 μl of anti-CPC-1 antibody. Immunoprecipitated DNA was quantified by using real-time polymerase chain reaction with primer pairs. The primer pairs used are listed in Table S1. ChIP-quantitative PCR data were normalized by the input DNA and presented as a percentage of input DNA. Each experiment was independently performed at least three times.

Acknowledgements

We thank Yubo He for critically evaluating the manuscript.

References

Ahn, S.H., Kim, M., and Buratowski, S. (2004) Phosphorylation of serine 2 within the RNA polymerase II C-terminal domain couples transcription and 3′ end processing. *Mol Cell* **13**: 67–76.

Ames, B., Shigenaga, M., and Hagen, T. (1993) Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci U S A* **90**: 7915–7922.

Belden, W.J., Larrondo, L.F., Froehlich, A.C., Shi, M., Chen, C.-H., Loros, J.J., and Dunlap, J.C. (2007) The band mutation in Neurospora crassa is a dominant allele of RAS-1 implicating RAS signaling in circadian output. *Genes Dev* **21**: 1494–1505.

Borkovich, K.A., Alex, L.A., Yarden, O., Freitag, M., Turner, G.E., Read, N.D., *et al*. (2004) Lessons from the genome sequence of Neurospora crassa: tracing the path from genomic blueprint to multicellular organism. *Microbiol Mol Biol Rev* **68**: 1–108.

Bowman, E.A., and Kelly, W.G. (2014) RNA polymerase II transcription elongation and pol II CTD Ser2 phosphorylation: a tail of two kinases. *Nucleus* **5**: 224–236.

Buttke, T., and Sandstrom, P. (1994) Oxidative stress as a mediator of apoptosis. *Immunol Today* **15**: 7–10.

Chary, P., and Natvig, D. (1989) Evidence for three differentially regulated catalase genes in Neurospora crassa: effects of oxidative stress, heat shock, and development. *J Bacteriol* **171**: 2646–2652.

Colot, H.V., Park, G., Turner, G.E., Ringelberg, C., Crew, C. M., Litvinkova, L., *et al*. (2006) A high-throughput gene knockout procedure for Neurospora reveals functions for multiple transcription factors. *Proc Natl Acad Sci* **103**: 10352–10357.

Dong, Q., Wang, Y., Qi, S., Gai, K., He, Q., and Wang, Y. (2018) Histone variant H2A.Z antagonizes the positive effect of the transcriptional activator CPC1 to regulate catalase-3 expression under normal and oxidative stress conditions. *Free Radic Biol Med* **121**: 136–148.

Eick, D., and Geyer, M. (2013) The RNA polymerase II carboxy-terminal domain (CTD) code. *Chem Rev* **113**: 8456–8490.

Fridovich, I. (1995) Superoxide radical and superoxide dismutases. *Annu Rev Biochem* **64**: 97–112.

Glorieux, C., Zamocky, M., Sandoval, J.M., Verrax, J., and Calderon, P.B. (2015) Regulation of catalase expression in healthy and cancerous cells. *Free Radic Biol Med* **87**: 84–97.

Hansberg, W., de Groot, H., and Sies, H. (1993) Reactive oxygen species associated with cell differentiation in Neurospora crassa. *Free Radic Biol Med* **14**: 287–293.

Hautbergue, G., and Goguel, V. (2001) Activation of the cyclin-dependent kinase CTDK-I requires the heterodimerization of two unstable subunits. *J Biol Chem* **276**: 8005–8013.

He, Q., Cha, J., He, Q., Lee, H.-C., Yang, Y., and Liu, Y. (2006) CKI and CKII mediate the FREQUENCY-dependent phosphorylation of the WHITE COLLAR complex to close the Neurospora circadian negative feedback loop. *Genes Dev* **20**: 2552–2565.

Keogh, M.C., Podolny, V., and Buratowski, S. (2003) Bur1 kinase is required for efficient transcription elongation by RNA polymerase II. *Mol Cell Biol* **23**: 7005–7018.

Keogh, M.C., Kurdistani, S.K., Morris, S.A., Ahn, S.H., Podolny, V., Collins, S.R., *et al*. (2005) Cotranscriptional set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex. *Cell* **123**: 593–605.

Krogan, N., Kim, M., Tong, A., Golshani, A., Cagney, G., Canadien, V., *et al*. (2003) Methylation of histone H3 by Set2 in *Saccharomyces cerevisiae* is linked to transcriptional elongation by RNA polymerase II. *Mol Cell Biol* **23**: 4207–4218.

Li, J., Moazed, D., and Gygi, S. (2002) Association of the histone methyltransferase Set2 with RNA polymerase II plays a role in transcription elongation. *J Biol Chem* **277**: 49383–49388.

Li, B., Howe, L., Anderson, S., Yates, J., and Workman, J. (2003) The Set2 histone methyltransferase functions through the phosphorylated carboxyl-terminal domain of RNA polymerase II. *J Biol Chem* **278**: 8897–8903.

Li, B., Gogol, M., Carey, M., Lee, D., Seidel, C., and Workman, J.L. (2007) Combined action of PHD and chromo domains directs the Rpd3S HDAC to transcribed chromatin. *Science* **316**: 1050–1054.

Livak, K.J., and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2 (−Delta Delta C (T)) method. *Methods* **25**: 402–408.

Lledías, F., Rangel, P., and Hansberg, W. (1998) Oxidation of catalase by singlet oxygen. *J Biol Chem* **273**: 10630–10637.

McCord, J., and Fridovich, I. (1969) Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J Biol Chem* **244**: 6049–6055.

Michán, S., Lledías, F., Baldwin, J., Natvig, D., and Hansberg, W. (2002) Regulation and oxidation of two large monofunctional catalases. *Free Radic Biol Med* **33**: 521–532.

Ostapenko, D., and Solomon, M.J. (2005) Phosphorylation by Cak1 regulates the C-terminal domain kinase Ctk1 in *Saccharomyces cerevisiae*. *Mol Cell Biol* **25**: 3906–3913.

Qi, S., He, L., Zhang, Q., Dong, Q., Wang, Y., Yang, Q., *et al*. (2018) Cross-pathway control gene CPC1/GCN4 coordinates with histone acetyltransferase GCN5 to regulate catalase-3 expression under oxidative stress in Neurospora crassa. *Free Radic Biol Med* **117**: 218–227.

Qiu, H., Hu, C., and Hinnebusch, A.G. (2009) Phosphorylation of the pol II CTD by KIN28 enhances BUR1/BUR2 recruitment and Ser2 CTD phosphorylation near promoters. *Mol Cell* **33**: 752–762.

Rodriguez, C.R., Cho, E.J., Keogh, M.C., Moore, C.L., Greenleaf, A.L., and Buratowski, S. (2000) Kin28, the TFIIH-associated carboxy-terminal domain kinase, facilitates the recruitment of mRNA processing machinery to RNA polymerase II. *Mol Cell Biol* **20**: 104–112.

Ross, K., Kaldis, P., and Solomon, M. (2000) Activating phosphorylation of the *Saccharomyces cerevisiae* cyclin-dependent kinase, cdc28p, precedes cyclin binding. *Mol Biol Cell* **11**: 1597–1609.

Scandalios, J. (1997) Oxidative stress and defense mechanisms in plants: introduction. *Free Radic Biol Med* **23**: 471–472.

Scandalios, J. (2002) The rise of ROS. *Trends Biochem Sci* **27**: 483–486.

Scandalios, J. (2005) Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. *Braz J Med Biol Res* **38**: 995–1014.

Schliebs, W., Wurtz, C., Kunau, W.H., Veenhuis, M., and Rottensteiner, H. (2006) A eukaryote without catalase-containing microbodies: Neurospora crassa exhibits a unique cellular distribution of its four catalases. *Eukaryot Cell* **5**: 1490–1502.

Schumacker, P.T. (2006) Reactive oxygen species in cancer cells: live by the sword, die by the sword. *Cancer Cell* **10**: 175–176.

Smolle, M., Venkatesh, S., Gogol, M.M., Li, H., Zhang, Y., Florens, L., *et al*. (2012) Chromatin remodelers Isw1 and Chd1 maintain chromatin structure during transcription by preventing histone exchange. *Nat Struct Mol Biol* **19**: 884–892.

Sogaard, T.M., and Svejstrup, J.Q. (2007) Hyperphosphorylation of the C-terminal repeat domain of RNA polymerase II facilitates dissociation of its complex with mediator. *J Biol Chem* **282**: 14113–14120.

Srivastava, R., and Ahn, S.H. (2015) Modifications of RNA polymerase II CTD: connections to the histone code and cellular function. *Biotechnol Adv* **33**: 856–872.

Sterner, D., Lee, J., Hardin, S., and Greenleaf, A. (1995) The yeast carboxyl-terminal repeat domain kinase CTDK-I is a divergent cyclin-cyclin-dependent kinase complex. *Mol Cell Biol* **15**: 5716–5724.

Stump, A.D., and Ostrozhynska, K. (2013) Selective constraint and the evolution of the RNA polymerase II C-terminal domain. *Transcription* **4**: 77–86.

Sun, G., Zhou, Z., Liu, X., Gai, K., Liu, Q., Cha, J., *et al*. (2016) Suppression of WHITE COLLAR-independent frequency transcription by histone H3 lysine 36 methyltransferase SET-2 is necessary for clock function in Neurospora. *J Biol Chem* **291**: 11055–11063.

Thomas, M.C., and Chiang, C.M. (2006) The general transcription machinery and general cofactors. *Crit Rev Biochem Mol Biol* **41**: 105–178.

Venkatesh, S., Smolle, M., Li, H., Gogol, M.M., Saint, M., Kumar, S., *et al*. (2012) Set2 methylation of histone H3 lysine 36 suppresses histone exchange on transcribed genes. *Nature* **489**: 452–455.

Viladevall, L., St Amour, C.V., Rosebrock, A., Schneider, S., Zhang, C., Allen, J.J., *et al*. (2009) TFIIH and P-TEFb coordinate transcription with capping enzyme recruitment at specific genes in fission yeast. *Mol Cell* **33**: 738–751.

Wagner, E.J., and Carpenter, P.B. (2012) Understanding the language of Lys36 methylation at histone H3. *Nat Rev Mol Cell Biol* **13**: 115–126.

Wang, Y., Dong, Q., Ding, Z., Gai, K., Han, X., Kaleri, F.N., *et al*. (2016) Regulation of Neurospora Catalase-3 by global heterochromatin formation and its proximal heterochromatin region. *Free Radic Biol Med* **99**: 139–152.

Waris, G., and Ahsan, H. (2006) Reactive oxygen species: role in the development of cancer and various chronic conditions. *J Carcinog* **5**: 14.

Xiao, T., Hall, H., Kizer, K.O., Shibata, Y., Hall, M.C., Borchers, C.H., and Strahl, B.D. (2003) Phosphorylation of RNA polymerase II CTD regulates H3 methylation in yeast. *Genes Dev* **17**: 654–663.

Xu, H., Wang, J., Hu, Q., Quan, Y., Chen, H., Cao, Y., *et al*. (2010) DCAF26, an adaptor protein of Cul4-based E3, is essential for DNA methylation in Neurospora crassa. *PLoS Genet* **6**: e1001132.

Yamashita, K., Shiozawa, A., Watanabe, S., Fukumori, F., Kimura, M., and Fujimura, M. (2008) ATF-1 transcription factor regulates the expression of ccg-1 and cat-1 genes in response to fludioxonil under OS-2 MAP kinase in Neurospora crassa. *Fungal Genet Biol* **45**: 1562–1569.

Yang, S., Li, W., Qi, S., Gai, K., Chen, Y., Suo, J., *et al*. (2014) The highly expressed methionine synthase gene of Neurospora crassa is positively regulated by its proximal heterochromatic region. *Nucleic Acids Res* **42**: 6183–6195.

Zamocky, M., Furtmuller, P.G., and Obinger, C. (2008) Evolution of catalases from bacteria to humans. *Antioxid Redox Signal* **10**: 1527–1548.

Zhao, Y., Shen, Y., Yang, S., Wang, J., Hu, Q., Wang, Y., and He, Q. (2010) Ubiquitin ligase components Cullin4 and DDB1 are essential for DNA methylation in Neurospora crassa. *J Biol Chem* **285**: 4355–4365.

Zhou, Z., Liu, X., Hu, Q., Zhang, N., Sun, G., Cha, J., *et al*. (2013) Suppression of WC-independent *frequency* transcription by RCO-1 is essential for *Neurospora* circadian clock. *Proc Natl Acad Sci* **110**: E4867–E4874.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Supplementary Table 1.