Chemical inhibition of the TFIIH-associated kinase Cdk7/Kin28 does not impair global mRNA synthesis

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The process of gene transcription requires the recruitment of a hypophosphorylated form of RNA polymerase II (Pol II) to a gene promoter. The TFIIH-associated kinase Cdk7/Kin28 hyperphosphorylates the promoter-bound polymerase; this event is thought to play a crucial role in transcription initiation and promoter clearance. Studies using temperature-sensitive mutants of Kin28 have provided the most compelling evidence for an essential role of its kinase activity in global mRNA synthesis. In contrast, using a small molecule inhibitor that specifically inhibits Kin28 *in vivo*, we find that the kinase activity is not essential for global transcription. Unlike the temperature-sensitive alleles, the small-molecule inhibitor does not perturb protein–protein interactions nor does it provoke the disassociation of TFIIH from gene promoters. These results lead us to conclude that other functions of TFIIH, rather than the kinase activity, are critical for global gene transcription.

chemical genetics | CTD kinase | TFIIH disassembly

TFIIH, a 10-subunit complex with many resident enzymatic activities, is essential for transcription by RNA polymerase II (Pol II) (1–5). TFIIH along with Pol II and several other multisubunit complexes assembles into a preinitiation complex (PIC) at the promoters of protein-coding genes (1–3). A subunit of TFIIH with helicase activity unwinds DNA and promotes the formation of a transcriptionally competent "open" complex (5–10). Concomitant with the open complex formation, the TFIIH-associated kinase (Cdk7) phosphorylates the C-terminal domain (CTD) of Rpb1, the largest subunit of Pol II (5, 11–13). The CTD consists of multiple YSPTSPS heptapeptide repeats; the number of repeats increases with increasing complexity of the organism (26 or 27 in budding yeast, 52 in humans). Cdk7, known as Kin28 in *Saccharomyces cerevisiae*, specifically phosphorylates the fifth residue (Ser-5) of the heptad repeat (14–17). This phosphorylation event is thought to disrupt stable interactions between CTD and PIC components, thereby permitting polymerase to escape from the promoter and engage in productive transcript elongation (14, 18, 19). Ser-5 phosphorylation also serves as a signal for binding of the mRNA-capping complex as well as the histone methyltransferase Set1 to the early elongating polymerase (20–23).

The mechanistic importance of Cdk7/Kin28 mediated Ser-5 phosphorylation in promoter escape and transcript elongation is widely accepted (1–3, 10, 12, 24–27). However, *in vitro* studies using catalytically inactive mutants or broad-spectrum kinase inhibitors show conflicting dependence on kinase function for mRNA synthesis (5, 11, 18, 19, 28–31). The role of the CTD itself has been questioned by reports in which Pol II lacking the entire CTD is capable of efficient transcription *in vitro* (32–34). And yet, the CTD as well as Cdk7/Kin28 are essential for cellular viability. Thus, *in vitro* experiments have failed to fully define the physiological role of Cdk7/Kin28 in modulating Pol II-dependent transcription. Because Cdk7/Kin28 kinase is required for viability, the majority of *in vivo* studies have relied primarily on temperature-sensitive (ts) alleles of this kinase. At nonpermissive temperature, a loss of global CTD Ser-5 phosphorylation correlates with a rapid shutdown of Pol II transcription (4, 35, 36). Conversely, a recently identified mutation that attenuates Kin28 kinase activity had no effect on transcription of a few genes that were examined (17). However, because of the central role of the kinase in cellular viability, it is possible that the kinase-attenuated strain may have gained additional fortuitous mutations elsewhere in the genome or activated redundant adaptive pathways that permit cellular survival. Similar caveats apply to mutations in TFIIH subunits that attenuate Cdk7 function but do not impair mRNA synthesis (29, 37). Thus, the extent to which Cdk7/Kin28 contributes to global mRNA synthesis *in vivo* remains extremely controversial.

To investigate the role of Kin28 kinase activity in mRNA synthesis and to avoid adaptive changes in attenuated strains we applied a chemical–genetic strategy to rapidly and reversibly inhibit Kin28 *in vivo* (38–41). This strategy utilizes cell-permeable analogs of a kinase inhibitor PP1 to specifically inhibit engineered target kinases and not perturb the function of other ATP-binding proteins. A bulky "gatekeeper" residue in the ATP-binding pocket of the desired kinase is genetically replaced with a residue bearing a smaller side chain, typically an alanine or glycine. The engineered protein is then able to accommodate the analog as well as ATP, and, at low micromolar concentrations, the analog competitively inhibits ATP binding *in vivo* and blocks cellular enzyme function within minutes (38, 40). This rapid and potent inhibition of the targeted enzyme prevents long-term adaptive changes and does not perturb cellular localization or disrupt protein–protein interactions of target proteins. The analog-sensitive allele of Kin28 (Kin28as) was shown to be sensitive to 1-NA-PP1 *in vivo* and *in vitro* (41). However, chromatin immunoprecipitation analysis indicated that chemical inactivation of Kin28 led to only a modest reduction in polymerase occupancy across the constitutively expressed genes *PMA1* and *ADH1* (41). This observation is inconsistent with the global shutdown of mRNA synthesis upon thermal inactivation of Kin28ts. To determine whether the defect in transcription is more evident at other genes, we explored the consequences of kinase inhibition on genome-wide mRNA synthesis. Both constitutive and inducible effects were examined, and results were compared with those obtained from thermally inactivated

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Abbreviations: Pol II, RNA polymerase II; CTD, C-terminal domain of the largest subunit of Pol II; PIC, preinitiation complex; Cdk7/Kin28, yeast cyclin-dependent kinase; Kin28as, analog-sensitive allele of Kin28; Kin28ts, temperature-sensitive allele(s) of Kin28.

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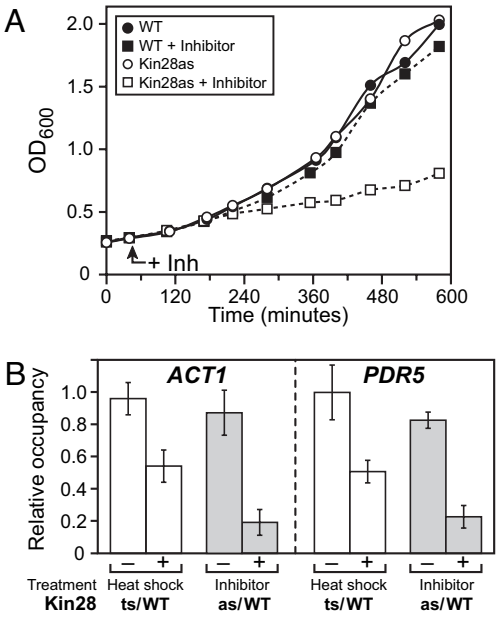

Fig. 1. 1-NA-PP1 inhibits Kin28as kinase activity *in vivo*. (*A*) Growth curves of Kin28as and isogenic WT strains in YPD with 2 μM 1-NA-PP1 or DMSO (solvent control) added 45 min after inoculation (arrow). (*B*) ChIP analysis of CTD-Ser-5 phosphorylation at *ACT1* and *PDR5* promoters. DNA immunoprecipitated with α-Ser-5 monoclonal antibody (H14) was quantitated and normalized by calculating fold enrichment over nontranscribed DNA in chromosome VI telomeric region. For each treatment, the normalized quantities for mutant strain were divided by normalized quantities for the respective isogenic WT strain. White bars represent this normalized ratio of Kin28ts3 grown at 25°C or 1 hour after shift to 37°C. Similarly, gray bars represent normalized ratio of Kin28as treated for 1 hour with 1-NA-PP1 or DMSO.

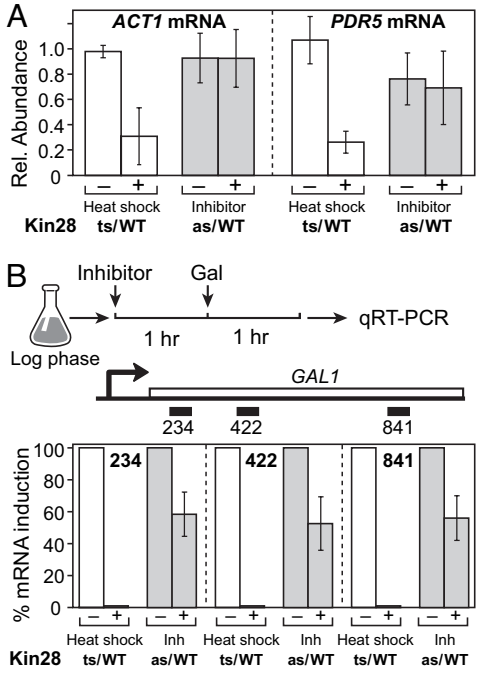

Fig. 2. Inhibition of Kin28 kinase activity has minimal effect on steady-state or inducible transcription. (*A*) Quantitative RT-PCR measure of the steady-state levels of *ACT1* and *PDR5* mRNA. White bars represent the ratio of transcript abundance in Kin28ts strains that are grown at 25°C or 1 hour after switch to nonpermissive temperature, whereas gray bars depict the ratio of transcripts in Kin28as 1 hour after treatment with 5 μM 1-NA-PP1 or DMSO. Approximate position of the center of qPCR products are as shown in Fig. 5 and SI Fig. 7. (*B*) Kin28as (gray) was grown to early log phase in YP-raffinose, and the culture was split and treated with either 10 μM 1-NA-PP1 or DMSO. One hour after treatment, galactose was added to the medium, and cultures were grown for an additional hour before RNA was harvested for qRT-PCR. Kin28ts (white) was grown similarly, and the cultures were switched to nonpermissive temperature or retained at permissive temperature for 1 hour before addition of galactose. The reference treatment (DMSO or permissive temperature) was set to equal 100% induction. Approximate positions of the center of qRT-PCR products relative to the *GAL1* start codon are shown in the diagram.

Kin28ts alleles. We find that Kin28 enzymatic function is not essential for global gene transcription, although it is necessary for efficient 5′ capping of mRNA transcripts. The differences between the temperature-sensitive versus analog-sensitive strains are explained by our observation that at nonpermissive temperatures two widely used Kin28ts alleles lead to the dissociation of the TFIIH complex from promoters, whereas the chemically inactivated allele retains TFIIH at promoters. Thus, it is the TFIIH complex and likely the resident helicase activity that is essential for the early stages in transcription.

Results and Discussion

Specific Inhibition of Kin28 Activity *in Vivo*. The 1-NA-PP1 effectively decreased the growth of the strain harboring the analog-sensitive Kin28as allele while displaying no detectable effect on the isogenic WT strain [Fig. 1*A* and supporting information (SI) Fig. 6]. Our data are consistent with previous observations that this analog dramatically inhibits kinase activity *in vivo* when added at 50-fold molar excess over IC_{50} (38). The 1-NA-PP1 was previously shown to inhibit kinase activity *in vitro* and decrease the bulk CTD phosphorylation *in vivo* (41); however, to determine whether the analog-inhibited Kin28as associated with highly active promoters, we measured the levels of CTD (Ser-5) phosphorylation at *ACT1* and *PDR5* by chromatin immunoprecipitation (ChIP). We find that Ser-5 phosphorylation was significantly diminished in the presence of 5 μM inhibitor (Fig. 1*B*). This inhibition was achieved within 20 min of adding 1-NA-PP1 and was sustained for at least 72 hours (data not shown). This analog is known to inactivate similarly engineered yeast kinases within 10 min (38, 40, 42). To compare the efficiency of kinase inhibition, we also performed ChIP experiments with Kin28ts3, a widely used temperature-sensitive allele. The results indicate that at the nonpermissive temperature, Ser-5 levels at *PDR5* and *ACT1* decrease in the Kin28ts3 strain; however, the inhibitory analog (1-NA-PP1) is far more effective at blocking Ser-5 phosphorylation by Kin28as (Fig. 1*B*). The residual Ser-5 phosphorylation in both Kin28ts3 and Kin28as strains may arise because of the action of other kinases (41) or because of incomplete inhibition. Moreover, the ability of 1-NA-PP1 to completely block Kin28as from phosphorylating a different target (Gal4) *in vivo* has been independently demonstrated (43). Thus, multiple independent lines of evidence show that 1-NA-PP1 is capable of rapidly and potently inhibiting Kin28as function *in vivo*.

Inhibition of Kin28 Does Not Inhibit Transcription. Having demonstrated that 1-NA-PP1 is a potent inhibitor of Kin28as kinase activity even when it is associated with active promoters, we examined the consequences of kinase inhibition on transcription of specific genes as well as on global transcriptome profiles. First, we determined the effect of either chemical inhibition (Kin28as) or heat inactivation (Kin28ts3) on steady state expression of *ACT1* and *PDR5*. Quantitative RT-PCR (qRT-PCR) measurements show a 5-fold decrease in mRNA levels of both genes in a Kin28ts3 strain after one hour at nonpermissive temperature (Fig. 2*A*). However, potent chemical inhibition of the kinase activity has no consequence on the transcript abundance of either gene (Fig. 2*A*).

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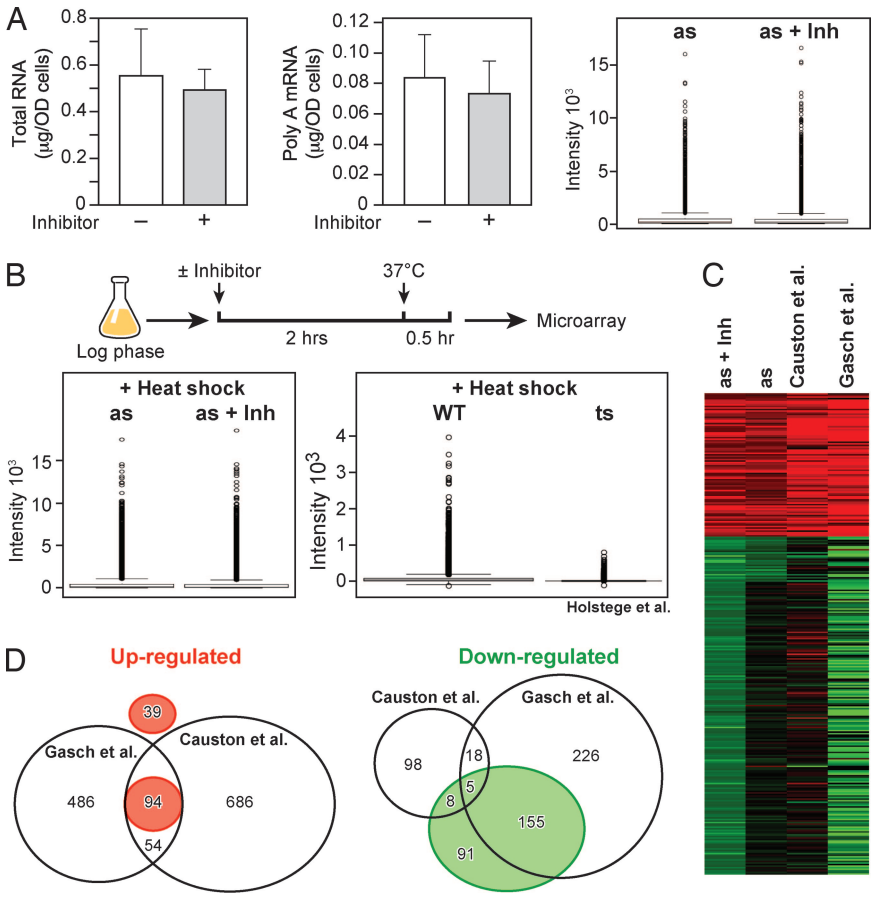

Fig. 3. Kin28as inhibition shows no global defect in transcription. (*A*) The 1-NA-PP1 (inhibitor) has negligible effect on total RNA yields (*Left*) or polyA mRNA yields (*Center*) in Kin28as strains. RNA was isolated from cultures treated with 10 μM 1-NA-PP1 (gray bars) or the solvent DMSO (white bars). Intensity box plots (*Right*) of three averaged microarrays of Kin28as strains treated for 2 hours with DMSO or 1-NA-PP1. Each circle represents a specific gene, with intensities indicating the abundance of a given mRNA transcript. The distribution of intensities indicates the levels of polyadenylated mRNAs. (*B*) Schematic diagram of microarray experiment (*Upper*) and box plots of the transcriptome of Kin28as strains treated for two hours with control solvent (DMSO) or with 1-NA-PP1 (Inh) and then shifted to 37°C for 30 min (*Lower Left*). (*Lower Right*) Box plot rendition of the Kin28ts3 and WT heat-shock expression data from Holstege *et al.* (36). (*C*) Heat map diagram of differentially expressed genes in response to heat shock. Genes that were differentially expressed in response to heat shock in Kin28as cells treated with inhibitor (first column) or DMSO (second column) were identified and compared with each other and published data sets of heat shock response (46, 47). Similar genes were up-regulated (red) with and without Kin28 inhibition, whereas more genes were down-regulated (green) in response to Kin28 inhibition. (*D*) Venn diagram of heat shock response. Genes identified as up-regulated in both DMSO and inhibitor treatment (*Left*) in this study (red circles) overlap with heat shock-responsive genes common for two published heat shock data sets; probes for the remaining 39 up-regulated transcripts were not present in the published data sets (outside red circle). (*Right*) Overlap of genes identified as down-regulated upon heat shock, in chemically inhibited Kin28as strain (green) and in published data sets.

To rule out indirect effects of the inhibitor on mRNA stability we tested the ability of the inhibited Kin28 strains to rapidly induce gene expression in response to extrinsic signals, like galactose and heat shock. The galactose-responsive expression of *GAL1* was examined because this robust response is abolished in heat inactivated Kin28ts3 strain (35, 44, 45). Furthermore, as noted above, the inhibitor eliminates galactose-responsive phosphorylation of Gal4 by Kin28as (43). In agreement with previous reports, quantitative RT-PCR measurements show that *GAL1* mRNA is not detected in heat-inactivated Kin28ts3 (Fig. 2*B*). In contrast, inhibition of Kin28as affects *GAL1* induction only modestly (Fig. 2*B*). The modest decrease occurs at a relatively early stage in transcription because PCR probes centered at ≈234, 422, and 841 nucleotides downstream of the start codon show similar levels of transcript abundance.

To determine the consequences of chemical inhibition of Kin28 on global mRNA synthesis, we first measured total RNA content of cells treated for 2 h with 1-NA-PP1 (Fig. 3*A*). The effect of inhibitor on cellular growth is first apparent at this time interval (Fig. 1*A*). The subtle reduction in total RNA levels is not surprising, because the majority of cellular RNA consists of ribosomal RNA and is not synthesized by Pol II. However, contrary to global shutdown of transcription in thermally inactivated Kin28ts strains (35, 36), bulk polyA-bearing RNA showed only a modest decrease in abundance upon chemical inhibition of the kinase (Fig. 3*A Center*). The identity and abundance of the polyadenylated RNA was examined by using yeast arrays (Affymetrix, Santa Clara, CA). The levels of all mRNAs in the transcriptome are displayed in a box plot wherein each circle represents a specific gene transcript and the intensity level is indicative of transcript abundance. As shown in Fig. 3*A Right*, the global distribution of transcript abundance is not significantly altered upon treatment with the inhibitor. Moreover, consistent with previously reported modest decrease in Pol II occupancy in the coding regions of *ADH1* and *PMA1* (41), we find that the mRNA abundance of these two genes also is proportionately down-regulated upon Kin28 inhibition ($P < 0.005$). This correlation provides independent validation of the transcript levels reported by the array. Importantly, these results compellingly argue against a global defect in mRNA synthesis upon inhibition of Kin28 kinase activity.

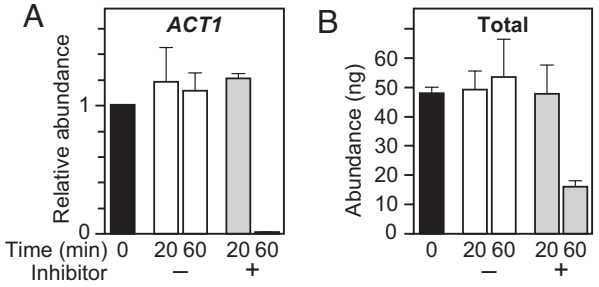

Fig. 4. Kin28as inhibition reduces transcript capping. Kin28as cultures were grown to OD_{600} of 0.3 and treated with inhibitor or DMSO. Cells were harvested at time 0 (black bars) and at 20 and 60 min after treatment (white bars for DMSO treatment and gray bars for inhibitor). Five micrograms of total RNA was used for immunoprecipitation experiments with H20, an anti-5′ cap monoclonal antibody. (*A*) Quantitative RT-PCR using *ACT1*-specific primers on the immunoprecipitated RNA. (*B*) The quantification of total immunoprecipitated RNA after treatment with the inhibitor or DMSO. Averages of three independent experiments are shown, and the standard deviations are displayed as error bars.

Finally, we tested whether cells with significantly impaired Kin28 activity can mount the necessary global transcriptional response to survive a major environmental perturbation. The genome-wide heat-shock response of the Kin28as strains that were grown in the presence of 1-NA-PP1 for 2 h was examined (Fig. 3*B*). In contrast to the near-complete shutdown of gene expression reported for Kin28ts3 under heat shock (36) genome-wide mRNA synthesis is not impaired in the chemically inhibited Kin28as strain upon heat shock (Fig. 3*B*). This experiment eliminates the possibility that elevated temperature, in addition to kinase inhibition, is required for the global shutdown of transcription observed in the Kin28ts strain. Furthermore, the heat shock profile of the chemically inhibited Kin28as strain closely matched the gene expression signatures of WT strains that were subjected to heat shock (46, 47) (Fig. 3 *C* and *D* and SI Data Set 1).

In essence, chemical inhibition of Kin28 kinase activity resulted in a dramatic decrease of CTD phosphorylation but showed no apparent defect in transcription initiation, promoter clearance, transcript elongation, no global reduction in mRNA steady-state levels and no global defect in the induced gene expression.

Kin28 Inhibition Correlates with Reduction in Capped Transcripts. CTD phosphorylation by Kin28 enhances 5′ capping of nascent transcripts (17, 21, 48). Similarly, we find that chemical inhibition of Kin28 kinase function leads to a severe reduction in 5′-capping of transcripts (Fig. 4). The capped transcripts from Kin28as cultures treated with the inhibitor (or DMSO) were immunoprecipitated with H20, a monoclonal antibody that binds the m^7G and trimethylG caps (see *SI Methods* for details). The abundance of m^7G capped *ACT1* and most cellular transcripts decreases dramatically by 60 min after Kin28 inhibition. The residual capped transcripts in Fig. 4*B* are most likely the relatively long-lived snRNA that bear trimethyl caps. The reduction in 5′ capped transcripts further validates the robust inhibition of Kin28as by 1-NA-PP1.

Kin28 Inactivation and TFIIH Destabilization. A possible explanation for the discrepancy between the transcriptional responses of a temperature-sensitive mutant and the analog-sensitive mutant of Kin28 is that upon heat inactivation, Kin28ts alleles are unable to retain protein interactions with other subunits of the TFIIH complex. The Tfb3 subunit of the Kin28-Ccl1-Tfb3 trimeric subcomplex is known to dissociate upon heat inactivation of

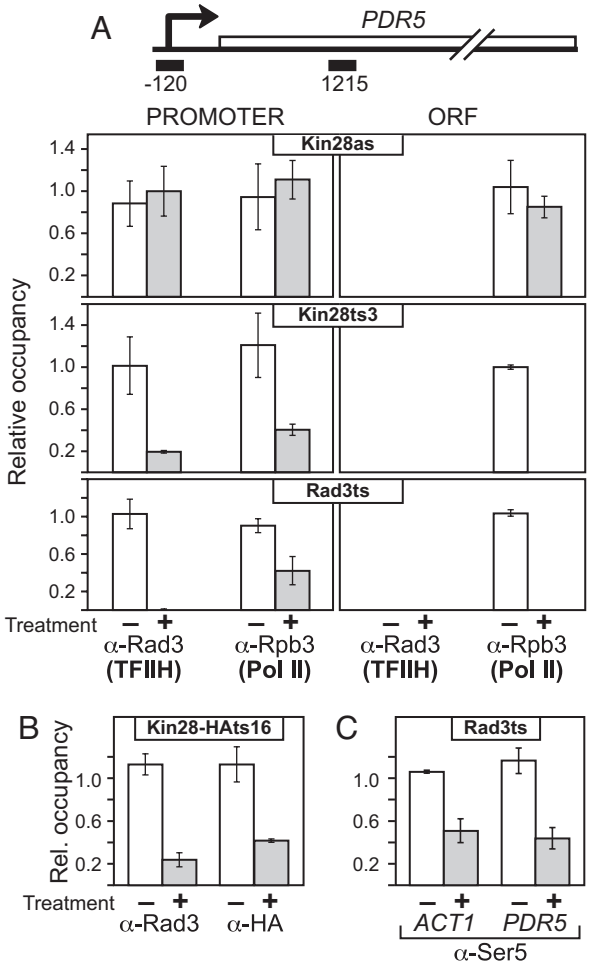

Fig. 5. TFIIH and Pol II retention upon chemical inhibition or heat inactivation. (*A*) ChIP analysis of TFIIH and Pol II occupancy at the *PDR5* promoter and ORF during chemical inhibition of Kin28as and heat inactivation of temperature-sensitive mutants. DNA was immunoprecipitated with antibodies against Rad3 or Rpb3 from Kin28as (*Top*), Kin28ts3 (*Middle*), or Rad3ts (*Bottom*) and isogenic WT strains. The Kin28as strain was treated for 1 hour with DMSO (white) or 5 μM 1-NA-PP1 (gray), whereas the temperature-sensitive strains were grown and subjected to ChIP analysis at 25°C (white) or 1 hour after shift to 37°C (gray). Immunoprecipitated DNA was normalized and quantified as in Fig. 1*B*. (*B*) TFIIH occupancy at the *PDR5* promoter in the Kin28ts16 strain. DNA immunoprecipitated with α-Rad3 and α-HA antibodies from Kin28-ts16-HA, and isogenic WT strains at 25°C or 1 hour after shift to 37°C was quantified by using qRT-PCR. Immunoprecipitated DNA was normalized and quantified as in Fig. 1*B*. (*C*) Levels of Ser-5-CTD phosphorylation at *PDR5* and *ACT1* promoters during heat inactivation of Rad3ts mutants. DNA immunoprecipitated with H14 antibody from Rad3-ts and isogenic WT strain at 25°C (white) or 1 hour after shift to 37°C (gray) was quantified by using qPCR. Immunoprecipitated DNA was normalized and quantified as in Fig. 1*B*.

Kin28ts (28, 49). The absence of this trimeric kinase subcomplex does not impair the ability of the residual seven-subunit core TFIIH complex to promote open complex formation and productive transcription *in vitro* (5, 28, 50). Similarly, inactivation of the Tfb3 homolog in *Schizosaccharomyces pombe* and Schwann cells does not impair global transcription *in vivo* (37, 51). By comparison, removal of the residual seven-subunit core complex results in cessation of transcription of most genes tested *in vitro* (5, 30). Inactivation of a helicase within this core subcomplex also leads to global inhibition of gene expression *in vivo* (52, 53).

To investigate whether the critical seven-subunit core complex remains associated with the PIC at the *ACT1* and *PDR5* promoters, we performed ChIP experiments using an antibody

against an integral subunit of the core complex, the Rad3/XPD helicase. In parallel, the level of Pol II retained at both genes was also monitored by ChIP (α-Rpb3 mAb). In the Kin28as strain, Rad3 and Pol II levels at *PDR5* or *ACT1* were comparable irrespective of the addition of the inhibitor (Fig. 5*A Top* and SI Fig. 7). In contrast, in the Kin28ts3 strain, heat inactivation led to a dramatic reduction of Rad3 occupancy and a decrease of Pol II at the promoters of *PDR5* (Fig. 5*A Middle*) as well as *ACT1* (SI Fig. 7). Furthermore, Pol II was not detected in the coding region (ORF) of either gene (Fig. 5*A* and SI Fig. 7). To further confirm these observations, TFIIH retention was examined in strains harboring Kin28ts16, another commonly used temperature-sensitive allele of Kin28 (Fig. 5*B*). In this case, Kin28 is epitope-tagged (HA) and ChIP experiments were performed by using the α-HA monoclonal antibody as well as the commercially available α-Rad3 antibody. Consistent with results of Fig. 5*A*, upon heat inactivation, both Rad3 and Kin28 dissociated from the *PDR5* promoter (Fig. 5*B*) as well as from the *ACT1* promoter (SI Fig. 7*B*).

We also performed the reciprocal experiment using a Rad3ts strain. At nonpermissive temperatures the Rad3ts mutant leads to disassembly of the TFIIH complex *in vitro* and rapidly eliminates global transcription *in vivo* (52, 54). Using ChIP analysis, we find that Rad3ts dissociates from both *PDR5* and *ACT1* promoters under nonpermissive conditions *in vivo* (Fig. 5*A Bottom* and SI Fig. 7). Moreover, the reduction of Rpb3 occupancy and Ser-5 phosphorylation (Fig. 5*C*) is remarkably similar to that observed with heat inactivated Kin28ts alleles, suggesting that the near-identical kinetics of transcriptional arrest exhibited by Rad3ts and Kin28ts strains result from the loss of TFIIH complex integrity. Taken together, the data strongly suggest that the defects in transcription seen in both Kin28ts strains as well as Rad3ts strain are attributable to the destabilization of TFIIH complex rather than the inactivation of the kinase activity.

The chemical–genetic approach described here has been used with great success to elucidate cellular functions of several different classes of kinases (38, 55), including the role of Cdc28 in *GAL1* induction (56). We used this approach to probe the conflicting reports on the role of Kin28 in mRNA synthesis. Our results strongly indicate that the kinase activity of Kin28 is not essential for promoter clearance, transcript elongation, or global mRNA synthesis. This conclusion is inconsistent with the widely held view that CTD phosphorylation by Kin28 is essential for mRNA synthesis. However, our results are in close agreement with a few genetic studies that arrived at a similar conclusion (21, 37, 51). Our data provide compelling evidence, because the arguments of long-term adaptive changes do not apply to the rapid and reversible chemical inhibition of Kin28 in living cells. Thus, the role of Kin28 as a CTD kinase is probably important for 5′ capping of transcripts and for enhancing the exchange of complexes that associate with Pol II during different stages of transcription. It is important to clarify however, that we focus on the role of Kin28 kinase activity rather than CTD phosphorylation in mRNA synthesis. It is possible that low-level phosphorylation of the CTD by Srb10 may suffice for the promoter release and transcript elongation by Pol II (41).

Another intriguing finding is that steady state mRNA levels do not diminish despite the dramatic reduction of capped RNA (Fig. 3*A*). It is noteworthy that previous observations of instability of uncapped transcripts are based on studies with temperature-sensitive capping mutants (57). As in the case of TFIIH, it is possible thermal inactivation of capping enzymes perturbs protein complexes and enhances mRNA instability

Finally, our data suggest that widely accepted models for the role of Kin28 kinase activity in transcription initiation and elongation need to be revised. Moreover, as new substrates for Kin28 are identified, additional roles of Kin28 in cellular function should be investigated.

Methods

Strains and Growth Conditions. Kin28as, Kin28ts16 (54), Kin28ts3 (35), Rad3ts (52), and their respective isogenic WT strains were grown in rich medium with raffinose or dextrose as a carbon source. *GAL1* induction experiments were performed by addition of galactose (2% final concentration) to cultures grown in yeast peptone raffinose medium.

RNA Preparation, Microarray Analysis, and Quantitative PCR. RNA was isolated by using the hot phenol method. Microarray experiments and quantitative RT-PCR were performed by using standard methods as described in *SI Methods*. Affymetrix GCOS version 1.2 was used to perform a local normalization based on Poly-A spiked in controls. Additional statistical analysis was performed with R and Bioconductor.

ChIP and RNA Immunoprecipitation. ChIP was performed as described (58) with modifications detailed in *SI Methods*. RNA immunoprecipitation was performed as in (59) with modifications detailed in *SI Methods*. H14 antibody (MMS-134R) was purchased from Covance, Rad 3 (sc-11963) and HA (sc-7392) antibodies were purchased from Santa Cruz Biotechnology, and remaining antibodies were gifted as described in Acknowledgments.

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