Bur1 kinase places new phosphorylation marks in RNA pol II CTD in the 3‘ region of genes

Department of Molecular Epigenetics

Chemical-genomic dissection of the CTD code.

Tietjen RJ, Zhang DW, Rodríguez-Molina JB, White BE, Akhtar MS, Heidemann H, Li X, Chapman RD, Shokat K, Keles S, Eick D & Ansari AZ (2010) *Nature Structural and Molecular Biology,* 17, 1154-1161

Post-translational modifications of the carboxyl-terminal domain (CTD) of the largest subunit of RNA polymerase II (Rpb1) specify a code that is deciphered by proteins involved in RNA biogenesis. The CTD is comprised of a repeating heptapeptide ($Y_1S_2P_3T_4S_5P_6S_7$). Recently, we could show that phosphorylation of Serine7 is important for co-transcriptional processing of two snRNAs in mammalian cells (1,2). Subsequently, we identified Kin28/Cdk7 (yeast/mammals), a subunit of the evolutionarily conserved TFIIH complex, as Ser7 kinase (3). Kin28/Cdk7 phosphorylates Ser5 and Ser7 residues at gene promoters *in vivo and in vitro* (3,4).
Here we have analyzed the distribution of CTD marks Ser5-P, Ser7-P and Ser2-P genome wide with the help of genetically engineered kinases, which can specifically be inhibited by small molecules. We identified the kinase Bur1, which phosphorylates CTD specifically at residue Ser7 in the 3’ region of genes.

(1) Chapman, R.D., Heidemann, M., Albert, T.K., Mailhammer, R., Flatley, A., Meisterernst, M., Kremmer, E. and Eick, D. (2007)
Transcribing RNA polymerase II is phosphorylated at CTD residue serine-7. *Science*, 318, 1780-1782.

(2) Egloff, S., O'Reilly, D., Chapman, R.D., Taylor, A., Tanzhaus, K., Pitts, L., Eick, D. and Murphy, S. (2007)
Serine-7 of the RNA polymerase II CTD is specifically required for snRNA gene expression. *Science*, 318, 1777-177

(3) Akhtar, M.S., Heidemann, M., Tietjen, J., Zhang, D., Chapman, R.D., Eick, D., Ansari, A.Z. (2009)
TFIIH kinase places bivalent marks on the carboxyl-terminal domain of RNA polymerase II. *Molecular Cell*, 34, 387-393

(4) Boeing, S., Rigault, C., Heidemann, M., Eick, D., and Meisterernst, M. (2010)
RNA PII CTD SER-7 phosphorylation is established in a mediator dependent fashion. *J. Biol. Chem.* 285, 188-196.

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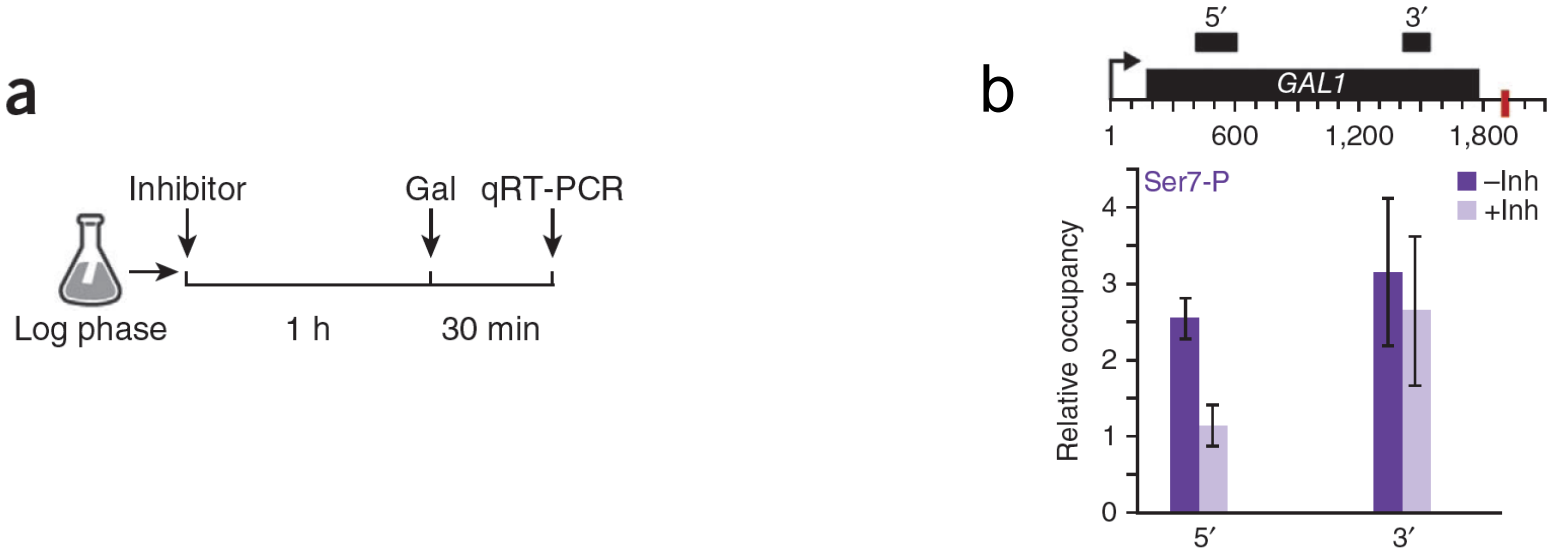

Promoter-distal Ser7-P marks are not remnants of promoter-proximal Kin28 phosphorylation. (a) Ser7-P marks within the coding region are not remnants of phosphorylation at the promoter. The Kin28as + Srb10as strain was treated for 60 min with DMSO or the inhibitors (inh) 1-NA-PP1/1-NM-PP1 under noninducing conditions. The GAL genes were induced for 30 min, and ChIP-qPCR was performed against the 5′ and 3′ ends of the GAL1 gene (black bars, primer location). **(b)** Upon inhibition, Ser7-P was significantly reduced at the 5′ end (P = 0.039) but not the 3′ end (P = 0.15) of GAL1.

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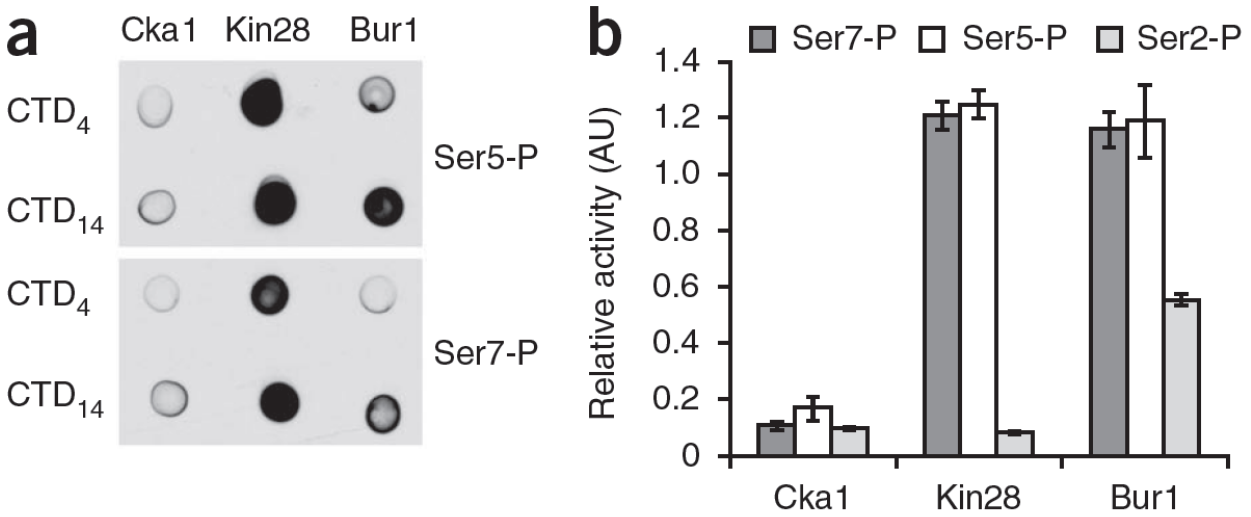

Bur1 kinase directly phosphorylates Ser7. (a) Kinase assay using a GST-CTD4 and GST-CTD14 substrates and purified Cka1, Kin28 and Bur1 kinases. The blots were probed with antibodies against Ser5-P (top) and Ser7-P (bottom). **(b)** ELISA of GST-CTD4 phosphorylated by purified yeast kinases and probed with antibodies against Ser7-P (dark gray bars), Ser5-P (white bars) and Ser2-P (light gray bars).