HelmholtzZentrum münchen
German Research Center for Environmental Health

Scientific Highlight April 2009

☐ co-ordinated with the Director of the Institute / Head of Department

Institute / Independent Department / Clinical Co-operation Group / Junior Research Group:
K.Molbi

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Person to contact for further enquiries:
Dirk Eick, eick@helmholtz-muenchen.de, 089-7099512

Title of the Highlight:
CTD kinase for Serine 7 identified

Keywords:
RNA Polymerase II, transcription, gene expression, epigenetics, chromatin

Central statement of the Highlight in one sentence:
Identification of the kinase for RNA Polymerase II carboxy-terminal domain Serine 7 phosphorylation in yeast and mammals

Text of the Highlight:
Gene expression is not just regulated by binding of the enzyme RNA polymerase II to the gene locus to which it is recruited, but also during the phase of active transcription from DNA into RNA. During this phase, parts of the newly synthesized RNA may be removed and the remaining sequences combined into new RNA message. This 'splicing' of RNA occurs during gene transcription, and in extreme cases, can produce RNAs coding for several thousand different proteins from a single gene. But what is the mechanism that permits this advance in gene usage? The RNA polymerase II has developed a structure composed of repeats of a 7 amino-acid sequence. In humans this structure – termed "carboxy-terminal domain" or CTD – is composed of 52 such heptad-repeats. It is placed exactly at the position where RNA emerges from RNA polymerase II. In less complex organisms the CTD is much shorter: a worm has 36 repeats, and yeast as few as 26, but many single-cell organisms and bacteria have never developed an obvious CTD structure.

Although the requirement of CTD for the expression of cellular genes in higher organisms is undisputed, the molecular details for the gene-specific maturation of RNAs is still largely enigmatic. We could recently show that phosphorylation of serine 7 in CTD is required for the processing and maturation of specific RNAs (Chapman et al., 2007; Egloff et al., 2007). Now we have identified with Kin28

and Cdk7 the cellular kinases responsible for serine 7 phosphorylation in yeast and mammals (Akhtar et al., 2009). Given the fundamental importance of CTD modifications for the regulation of gene expression, our research is essential for the understanding of complex diseases and for the development of new therapies.

Publications:

Chapman, R.D., Heidemann, M., Albert, T., Mailhammer, R., Flatley, A., Meisterernst, M., Kremmer, E., and Eick, D. (2007) RNA polymerase II CTD is phosphorylated at serine 7 during the transcription cycle. Science 318, 1780-1782

Egloff, S., O'Reilly, D., Chapman, R.D., Taylor, A., Tanzhaus, K., Pitts, L., Eick, D., and Murphy, S. (2007) A specific role for serine 7 of the pol II CTD in expression of human snRNA genes. Science 318, 1777-1780

Chapman, R.D., Heidemann, M., Hintermair, C., and Eick, D. (2008) Molecular evolution of RNA polymerase II CTD. Trends in Genetics 24, 289-296

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, in press

Taking account of the HMGU mission:

The internal HMGU co-operation partners with whom the Highlight was compiled, if appropriate:

CTD kinase for Ser7 identified.

Institute of Clinical Molecular Biology and Tumor Genetics

TFIIH kinase places bivalent marks on the carboxyl-terminal domain of RNA polymerase II

Akhtar, M.S., Heidemann, M., Tietjen, J., Zhang, D., Chapman, R.D., Eick, D., Ansari, A.Z. (2009) Molecular Cell, in press

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, phosphorylation of Serine7 was shown to be important for co-transcriptional processing of two snRNAs in mammalian cells (1,2). Here, we report that Kin28/Cdk7, a subunit of the evolutionarily conserved TFIIH complex, is a Ser7 kinase. The ability of Kin28/Cdk7 to phosphorylate Ser7 is particularly surprising because this kinase functions at promoters of protein-coding genes, rather than being restricted to promoter-distal regions of snRNA genes. Kin28/Cdk7 is also known to phosphorylate Ser5 residues of the CTD at gene promoters. Taken together, our results implicate the TFIIH kinase in placing bivalent Ser5 and Ser7 marks early in gene transcription. These bivalent CTD marks, in concert with cues within nascent transcripts, specify the co-transcriptional engagement of the relevant RNA processing machinery.

(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, 1777-1779.

CTD Ser7 is phosphorylated in budding yeast by Kin28 and in mammals by Cdk7

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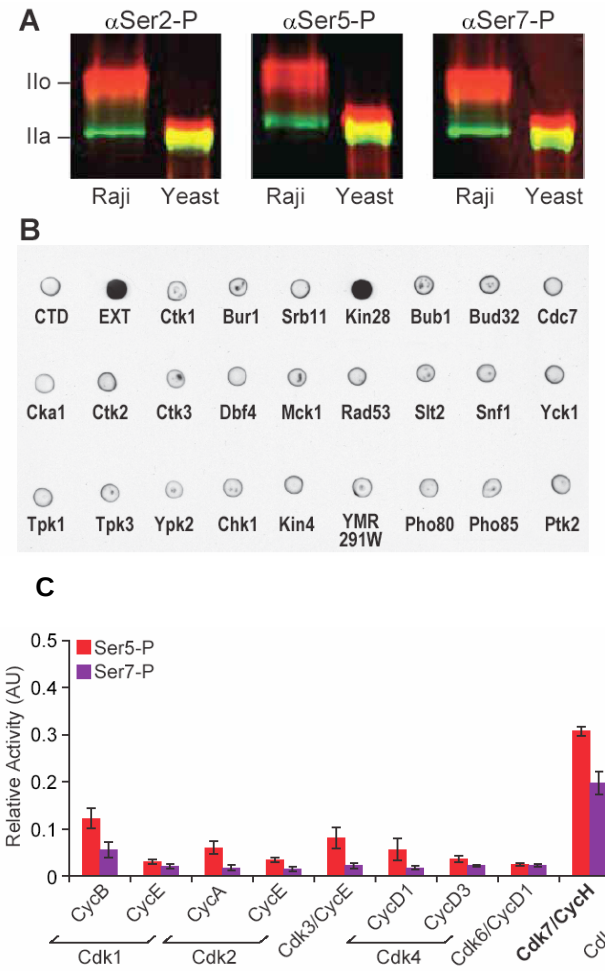

A. Western blot of proteins extracted from human B-cell line (Raji cells) and yeast. Dual labeling was performed with antibody to Rpb1 (green) and phospho-CTD (red). mAb Pol3/3 recognises an epitope in Rpb1 located outside the CTD. mAbs anti-Ser2-P, anti-Ser5-P and anti-Ser7-P recognize the phosphorylated Ser2, Ser5 and Ser7 of the CTD

B. Dot blot probing Ser7-P marks on GST-CTD phosphorylated by individually purified yeast nuclear kinases. GST-CTD unphosphorylated (GST) and phosphorylated by yeast cell extract (EXT) are used as negative and positive controls respectively.

C. Mammalian Cdk7 phosphorylates the Ser7 of Pol II CTD. ELISA of a synthetic CTD peptide (four repeats) phosphorylated by purified mammalian kinases and their corresponding cyclins were probed with αSer5-P (red) and αSer7-P (purple) antibodies. The measurements were taken in triplicate and the error bars correspond to SD.

Chemical inhibition of engineered Kin28 kinase(Kin28as) inhibits promoter proximal Ser5 and Ser7 phosphorylation

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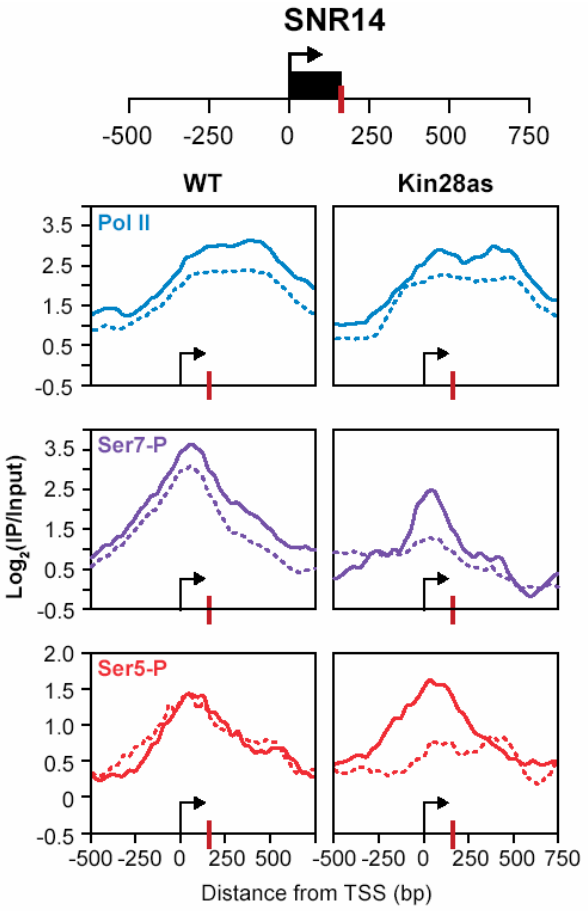

ChIP-chip profiles for the snRNA14 gene. The occupancy profiles of Pol II (blue), Ser7-P (purple) and Ser5-P (red) at SNR14 is shown. Uninhibited profiles are shown as solid lines and profiles in cells with chemically inhibited Kin28 are shown as dashed lines. TSS and 3' processing sites are marked by an arrow and a red bar respectively. All x-axes are shown as the distance in base pairs relative to the TSS and y-axes are shown on a log2 scale for ChIP-chip enrichment (IP/input).