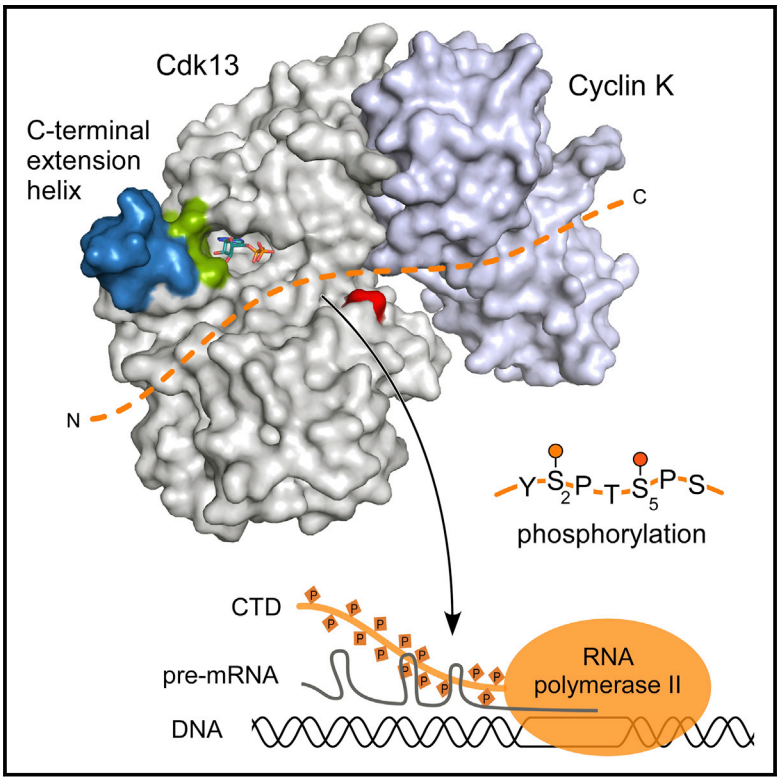
Article

Cell Reports

Structural and Functional Analysis of the Cdk13/Cyclin K Complex

Graphical Abstract



Authors

Ann Katrin Greifenberg, Dana Hönig, Kveta Pilarova, ..., Kanchan Anand, Dalibor Blazek, Matthias Geyer

Correspondence

geyer@uni-bonn.de

In Brief

Cyclin-dependent kinases regulate transcription through phosphorylation of RNA polymerase II. Greifenberg et al. report the structure of the human Cdk13/Cyclin K complex. Cdk13 contains a C-terminal extension helix that is a specific feature of transcription elongation kinases.

Highlights

- The crystal structure of the human Cdk13/Cyclin K complex
- Cdk13 phosphorylates Ser5 and Ser2 of the RNA polymerase II CTD
- The isomerase Pin1 does not change the phosphorylation specificity of Cdk13
- Cdk13 regulates genes involved in growth signaling pathways

Accession Numbers

5EFQ

Greifenberg et al., 2016, Cell Reports *14*, 320–331
January 12, 2016
http://dx.doi.org/10.1016/j.celrep.2015.12.025

OPEN ACCESS
CellPress

Cell Reports
Article

Structural and Functional Analysis of the Cdk13/Cyclin K Complex

Ann Katrin Greifenberg,[1,2] Dana Hönig,[2,3] Kveta Pilarova,[4] Robert Düster,[1,2] Koen Bartholomeeusen,[4] Christian A. Bösken,[2,3] Kanchan Anand,[1,5] Dalibor Blazek,[4] and Matthias Geyer[1,2,3,*]

[1]Institute of Innate Immunity, Department of Structural Immunology, University of Bonn, Sigmund-Freud-Strasse 25, 53127 Bonn, Germany
[2]Center of Advanced European Studies and Research, Group Physical Biochemistry, Ludwig-Erhard-Allee 2, 53175 Bonn, Germany
[3]Max Planck Institute of Molecular Physiology, Department of Physical Biochemistry, Otto-Hahn-Strasse 11, 44227 Dortmund, Germany
[4]Central European Institute of Technology (CEITEC), Masaryk University, 62500 Brno, Czech Republic
[5]Present address: EMBL Heidelberg, Structural and Computational Biology Programme, Meyerhofstrasse 1, 69117 Heidelberg, Germany
*Correspondence: geyer@uni-bonn.de
http://dx.doi.org/10.1016/j.celrep.2015.12.025

SUMMARY

Cyclin-dependent kinases regulate the cell cycle and transcription in higher eukaryotes. We have determined the crystal structure of the transcription kinase Cdk13 and its Cyclin K subunit at 2.0 Å resolution. Cdk13 contains a C-terminal extension helix composed of a polybasic cluster and a DCHEL motif that interacts with the bound ATP. Cdk13/CycK phosphorylates both Ser5 and Ser2 of the RNA polymerase II C-terminal domain (CTD) with a preference for Ser7 pre-phosphorylations at a C-terminal position. The peptidyl-prolyl isomerase Pin1 does not change the phosphorylation specificities of Cdk9, Cdk12, and Cdk13 but interacts with the phosphorylated CTD through its WW domain. Using recombinant proteins, we find that flavopiridol inhibits Cdk7 more potently than it does Cdk13. Gene expression changes after knockdown of Cdk13 or Cdk12 are markedly different, with enrichment of growth signaling pathways for Cdk13-dependent genes. Together, our results provide insights into the structure, function, and activity of human Cdk13/CycK.

INTRODUCTION

Higher organisms have evolved a unique structure in the RNA polymerase II (RNAPII) to coordinate transcription and the attendant processing of pre-mRNA molecules. The largest subunit Rpb1 of RNAPII contains a C-terminal extension, termed the C-terminal domain, or CTD, which is composed of multiple repeats of the hepta-sequence $Y_1S_2P_3T_4S_5P_6S_7$. The number of repeats varies between 26 in yeast and 52 in humans and is thought to correlate with the genomic complexity of the organism (Buratowski 2009; Corden 2013; Eick and Geyer, 2013). In addition, alterations from the consensus sequence occur in higher eukaryotes with a change in position 7 from serine to lysine prevailing in the distal part of the human CTD. The repetitive structure of the CTD is thought to act as a binding scaffold for transcription associated factors. The five hydroxyl group containing residues Tyr1, Ser2, Thr4, Ser5, and Ser7 are all susceptible to post-translational modifications (PTMs) combined with a *cis/trans* isomerization of the two intermediate prolines, Pro3 and Pro6. The reversible modifications by phosphorylation or *O*-linked glycosylation as well as acetylation and methylation of basic residues open a plethora of possible combinations of PTMs, often described as RNAPII CTD code.

Phosphorylation of the three serine residues within the CTD is tightly linked to the phases of RNAPII-mediated transcription (Adelman and Lis, 2012). At first, the unphosphorylated polymerase is recruited into the pre-initiation complex at open chromatin structures. In a simplified model, phosphorylation of Ser7 (pSer7) starts the transcription cycle until an additional pause step stalls the polymerase about 50 nucleotides downstream of the transcription start site (TSS). To overcome this promoter proximal pausing, Ser5 is phosphorylated, potentially in conjunction with Ser7, leading to the robust elongation of transcripts. On a molecular level, Ser7 phosphorylation primes the CTD for further modifications (Czudnochowski et al., 2012; St Amour et al., 2012), but, whereas Ser7 phosphorylation levels stay high during the transcription process, the pSer5 signal decreases steadily toward the poly-adenylation site by the action of phosphatases. Ser2 phosphorylation instead increases toward the transcription termination site (TTS), consistent with the recruitment of 3′ RNA-processing factors by pSer2 marks (Davidson et al., 2014). The phosphorylation signals are removed by phosphatases during the termination process, giving way for a new transcription cycle of the polymerase.

Cyclin-dependent kinases (CDKs) play major roles in the regulation of the cell cycle and transcription. Five mammalian CDKs have been described to date as transcription regulating kinases together with their corresponding cyclin subunits: Cdk7/Cyclin H as components of the general transcription factor TFIIH (Grünberg and Hahn, 2013), Cdk8/Cyclin C as components of the Mediator kinase module (Allen and Taatjes, 2015), Cdk9/Cyclin T1 or T2 that constitute the active form of the positive transcription elongation factor (P-TEFb) (Peterlin and Price, 2006), and Cdk12/Cyclin K and Cdk13/Cyclin K as the latest members of RNAPII CTD kinases (Bartkowiak et al., 2010; Blazek et al., 2011). A precise assignment of the various CDKs to the different