Title:

The interaction of CDK12/CrkRS with CYCLIN K1 is required for the phosphorylation of the C-terminal domain of RNA Pol II.

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Running Title:

CYCLIN K1 is required for CDK12/CrkRS kinase activity.

22 # Supplemental Data:
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24 ## Supplemental Methods
25
26 **MRM Mass Spectrometry.** We designed optimized MRM transitions for 3 signature peptides (3
27 transitions/peptide) for each of the CYCLIN L1, T1, K1 and K2 and CDK12, CDK9 and CDK11
28 based on our MS/MS data (Supplemental Table S2). Peak areas are proportional to peptide
29 amounts. MRM data were processed using MultiQuant v.1.0 (Applied Biosystems). Default
30 values for noise percentage and minimum peak height were used (40% and 50 CPS,
31 respectively). Manual inspection ensured that correct peaks were integrated for all samples.
32 Integrated peak area values for multiple transitions were summed (Microsoft Excel).
33 **Quantitative PCR**. Cells were lysed in Trizol (Invitrogen) and total RNA was prepared. The
34 samples were then treated with amplification grade DNAse I (Invitrogen). cDNA was generated
35 by reverse transcription (RT) with SuperScript III Reverse Transcriptase (Invitrogen)
36 Quantitative real-time RT-PCR was done as described previously and the relative expression
37 changes were determined with the 2-ΔΔCT method with GAPDH used as a normalization control
38 (2, 4). The primer sequences used are: CDK12:5'-GAAGAGAGTCGCCCTTACACAAAC-3',
39 5'-AATACATCCACAGCTCCAAACATC-3', CYCLIN K: 5'-CACCCAGGTTGTTTCTCC-3',
40 5'-CCTCACCTAAGGCAGCAG-3', GAPDH: 5'-AGAAGGCTGGGGCTCATTTG-3', 5'-
41 AGGGGCCATCCACAGTCTTC-'3.

Supplemental Results:

MRM Analysis of CDK/Cyclin complexes

To estimate the relative level of CDK or cyclin partners for a given cyclin or CDK we isolated ectopic and endogenous CDK12 and CDK9 complexes by immunoprecipitation. The protein complexes resolved by SDS-PAGE and each lane was cut into multiple slices corresponding to specific molecular weight ranges. The peptides within each gel slice were then analyzed by MRM. The reason behind this method of analysis is that we could not rule out the possibility that CYCLIN K2 (43 KDa) may be expressed in HEK239A cells because this cell line was not among the 570 RNA-seq libraries examined for CYCLIN K splice variants. The separation of the immunoprecipitated protein complexes by SDS-PAGE allows CYCLIN K1 (64 KDa) to be distinguished from CYCLIN K2 (43 KDa). The MRM analysis of CDK12 immunoprecipitates showed that CDK12 interacted primarily with CYCLIN K, with CYCLIN K1 specific and CYCLIN K1/K2 common peptide signatures found predominantly in the 65-100 KDa gel slices (Supplemental Fig. S1A). We did not detect any CYCLIN K2 specific peptide signatures in any of our CDK12 immunoprecipitates or significant levels of any CYCLINK peptides in the 40-53 KDa ranges (Supplemental Fig.S1A). This supports our observation that CYCLIN K1 is the predominant CYCLIN K isoform that interacts with CDK12. We were able to detect one peptide of CYCLIN L1 in both endogenous and ectopic CDK12 immunoprecipitates. However, the signal from this CYCLIN L1 peptide was ~15 fold lower and ~6 fold lower than the signal for CYCLIN K in the endogenous and ectopic expressed CDK12 immunoprecipitates, respectively. This suggests that CYCLIN L1 is a very minor cyclin partner of CDK12 at endogenous protein levels.

64 MRM analysis of endogenous and ectopic CDK9 complexes shows that CYCLIN T1 was
65 the primary cyclin interacting with CDK9 in HEK293A (Supplemental Fig. S1B). Low levels of
66 CYCLIN K1 and K1/K2 peptide signatures were observed in 3xFLAG-CDK9
67 immunoprecipitates in the 60-90 KDa gel slices suggesting that CYCLIN K1 may be a minor
68 partner of CDK9 when CDK9 is ectopically expressed (Supplemental Fig. S1B). Again, no
69 CYCLIN K2 peptide signatures were observed. The finding that the common CYCLIN K1/K2
70 MRM peptide signatures are predominantly found in molecular weight ranges consistent with the
71 molecular weight of CYCLIN K1 is further evidence that CYCLIN K1 is the predominant
72 isoform expressed in mammalian cells.

73 Studies have shown that cyclins can interact with multiple CDK partners (1, 3, 5, 7).
74 Therefore, we analyzed 3xFLAG tagged CYCLIN L1, T1 and K1 immunoprecipitates to look for
75 CDK12, CDK11 and CDK9 by MRM. In 3xFLAG-CYCLIN L1 immunoprecipitates, CDK11
76 peptide signatures were found in the 80-130 KDa range, consistent with the molecular weight of
77 CDK11^{p110} (Supplemental Fig. S2) (6). However, we were unable to detect CDK12 by MRM in
78 the 3xFLAG-CYCLIN L1 immunoprecipitates (Supplemental Fig. S2). In 3xFLAG CYCLIN T1
79 immunoprecipitates, only CDK9 peptide signatures were found (predominantly between 48-56
80 KDa) and these signals coincide with the expected molecular weight of range of both CDK9
81 isoforms (42 and 55 KDa) (Supplemental Fig. S2). MRM analyses of 3xFLAG-CYCLIN K1
82 immunoprecipitates showed the presence of CDK12 and CDK13/CDC2L5 peptide signatures
83 between the 180-250 KDa molecular weight gel slices, consistent with our IP-MS results
84 (Supplemental Fig. S2). One CDK9 peptide signature was detected within 48-56 KDa gel slice
85 but the signal from this one peptide was substantially lower than CDK12 and CDK13/CDC2L5
86 where we detected signals from 2 and 3 peptides, respectively. This suggests that CDK12 and

87 CDK13 are the primary partners of CYCLIN K1 and that CDK9 is a minor partner at

88 endogenous protein expression levels in HEK293A.

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90 **References:**

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116 <u>**Supplemental Figure Legends**</u>

117 **Supplemental Fig S1. Multiple Reaction Monitoring (MRM) analysis of**

118 **immunoprecipitated complexes.**

119 Immunoprecipitated protein complexes were separated by SDS-PAGE. The gel was cut into

120 approximate molecular weight ranges, subjected to in-gel trypsin digestion and the resulting

121 peptides were analyzed by MRM. Different shades of grey on the stacked columns represent

122 different peptides from the same protein used in the MRM assay. A minimum of two transitions

123 per peptide were summed. **A.** CYCLIN MRM analysis of endogenous CDK12 and 3xFLAG-

124 CDK12 protein complexes showed that CDK12 complexes contained CYCLIN K1 and not

125 CYCLIN K2. CYCLIN K1 and CYCLIN K2 were distinguished in this assay by CYCLIN K1

126 and CYCLIN K2 specific MRM signatures as well as by molecular weight. CYCLIN L1

127 appeared to be a minor CDK12 interacting protein using this assay. **B.** CDK MRM analysis of

128 3xFLAG-CYCLIN K1 and 3xFLAG-CYCLIN L1 protein complexes showed that CYCLIN K1

129 interacted predominantly with CDK12 and CDK13 and minimally with CDK9, while CYCLIN

130 L1 interacted only with CDK11 in this assay.

131

132 **Supplemental Fig. S2. Multiple Reaction Monitoring (MRM) analysis of CYCLIN**

133 **immunoprecipitated complexes.**

134 3xFLAG-CYCLIN K1, L1 and T1 complexes were immunoprecipitated from HEK293A cells

135 and proteins were analyzed by MRM as described in Supplemental Methods. **A** CDK MRMs of

136 3xFLAG-CYCLIN K1, 3xFLAG-CYCLIN L1 and 3xFLAG-CYCLIN T1 protein complexes

137 showed that CYCLIN K1 interacted predominantly with CDK12 and CDK13 and minimally

138 with CDK9, while CYCLIN L1 interacted only with CDK11, and CYCLIN T1 interacted only

139 with CDK9.

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141 **Supplemental Fig. S3. Quantitative RT-PCR analysis of CDK12 and CYCLIN K mRNA**

142 **expression in siRNA treated cells.**

143 Quantitative RT-PCR was performed as described in Supplemental Methods. **A**. CYCLIN K

144 mRNA expression was reduced between 60-70% by the CYCLIN K 3' UTR siRNA in

145 HEK293A cells stably expressing 3xFLAG-CDK12 when normalized against a mock transfected

146 control. **B**. CDK12 mRNA expression in the 3xFLAG-CDK12 stable cell line was typically 8-12

147 fold higher than the control cell line. Vertical error bars denotes the standard deviation in 3

148 replicates.

149

150 **Supplemental Table S1. Invitrogen STEALTHTM siRNA sequences used to knockdown the**

151 **expression of CDK9, CDK12 and CYCLIN K.**

152

153 **Supplemental Table S2. Multiple Reaction Monitoring (MRM) peptides and transitions**

154 **used for the detection of CDK and CYCLIN proteins.**