Cell PRESS
Open ACCESS
Cell Reports
Report

Cdk11-CyclinL Controls the Assembly of the RNA Polymerase II Mediator Complex

Julie Drogat,[1,4] Valérie Migeot,[1] Elise Mommaerts,[1] Caroline Mullier,[1] Marc Dieu,[2] Harm van Bakel,[3,5] and Damien Hermand[1,*]
[1]Namur Research College (NARC)
[2]Spectrometrie de Masse
The University of Namur, Namur 5000 Belgium
[3]Banting and Best Department of Medical Research, University of Toronto, Toronto, ON M5S 3E1, Canada
[4]Present address: Centre de Recherche en Cancérologie de Marseille (CRCM), UMR7258, 13273 Marseille, France
[5]Present address: Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1498, New York, NY 10029-6574, USA
*Correspondence: Damien.Hermand@fundp.ac.be
http://dx.doi.org/10.1016/j.celrep.2012.09.027

SUMMARY

The large Mediator (L-Mediator) is a general coactivator of RNA polymerase II transcription and is formed by the reversible association of the small Mediator (S-Mediator) and the kinase-module-harboring Cdk8. It is not known how the kinase module association/dissociation is regulated. We describe the fission yeast Cdk11-L-type cyclin pombe (Lcp1) complex and show that its inactivation alters the global expression profile in a manner very similar to that of mutations of the kinase module. Cdk11 is broadly distributed onto chromatin and phosphorylates the Med27 and Med4 Mediator subunits on conserved residues. The association of the kinase module and the S-Mediator is strongly decreased by the inactivation of either Cdk11 or the mutation of its target residues on the Mediator. These results show that Cdk11-Lcp1 regulates the association of the kinase module and the S-Mediator to form the L-Mediator complex.

INTRODUCTION

The process of messenger RNA (mRNA) transcription requires the interaction of a set of general transcription factors and a wide range of gene-specific factors. The Mediator complex is essential for the expression of nearly all RNA polymerase II (PolII)-dependent genes in *Saccharomyces cerevisiae* (Holstege et al., 1998; Thompson and Young, 1995), where it was first purified (Kelleher et al., 1990). Independently, genetic screens identified genes encoding Mediator subunits as suppressors of truncations of the carboxy-terminal domain (CTD) of the largest subunit of PolII, Rpb1 (Nonet and Young, 1989). Several lines of evidence suggest that Mediator bridges many DNA-binding transcription factors and PolII, which supports activator-dependent transcription (Conaway and Conaway, 2011). Furthermore, the Mediator enhances PolII recruitment and stabilizes complexes at the promoter (Cantin et al., 2003). The three-dimensional structure of a budding yeast Mediator reveals an extended complex that consists of three visually separate modules, termed head, middle, and tail, that form the small Mediator (S-Mediator) and an additional four subunits "kinase module" harboring the Cdk8 kinase that can associate with the core S-Mediator to form the large Mediator (L-Mediator) (Asturias et al., 1999; Dotson et al., 2000). There is compelling support for an ancient origin of the overall structure, although some metazoans include several isoforms of the kinase module and additional subunits, which may reflect specialization in genes regulation (Bourbon, 2008).

While the S-Mediator has a stimulatory effect on basal transcription (Myers et al., 1998; Spahr et al., 2003), early data showed that the L-Mediator instead represses transcription in vitro, and genome-wide analyses supported a negative role of the kinase module (Holstege et al., 1998). The current model proposes that S-Mediator interaction with the kinase module triggers a structural switch that prohibits interactions with PolII or simply sterically prevents it (Elmlund et al., 2006; Knuesel et al., 2009; Samuelsen et al., 2003). The structure of the L-Mediator suggests that the kinase module must be actively displaced to allow interaction of the S-Mediator with PolII, but the mechanism remains unknown. Besides this generally accepted model, it is also established that the kinase module plays important roles in gene activation in higher eukaryotes (Conaway and Conaway, 2011). Cdk8 functions as a coactivator in several transcriptional programs, including those governed by p53, the Wnt/β-catenin pathway, or the serum response network (Galbraith et al., 2010).

The tail module of the Mediator is critical for interactions with DNA-binding transcription factors (Myers et al., 1999). The broad structural variations in eukaryotic transactivators may be reflected in the evolutionary divergence of the tail module that is not visible in the fission yeast *Schizosaccharomyces pombe*, where some of its subunits were lost (Bourbon, 2008; Linder et al., 2008) (see also Figure S1B for a comparison of Mediator subunits composition in budding yeast, fission yeast, and human). Comparative genomic analyses suggested that Med24, Med27, and Med29 could be orthologous to budding

yeast Med5, Med3, and Med2 and belong to the tail module (Bourbon, 2008). Biochemical analyses in fission yeast and fly do not support this possibility (Linder et al., 2008; Sato et al., 2003a, 2003b), yet it was shown that *Drosophila* Med29 behaves as a tail module subunit, as it binds directly to transactivators (Garrett-Engele et al., 2002). Altogether, these data suggest that structural rearrangements have occurred during evolution.

Here, we have identified the Cdk11-Lcp1 complex in fission yeast as a key regulator of the assembly of the L-Mediator through the phosphorylation of Med4 and Med27. The metazoan PITSLRE kinase family (Cdk11) is characterized by the expression of two isoforms (p110 and p58) from the same mRNA due to the presence of a cell cycle-regulated internal ribosome entry site (Cornelis et al., 2000; Wilker et al., 2007; Yokoyama et al., 2008). Importantly, Cdk11 is a distinct kinase from Cdk19 that closely resembles Cdk8 and was sometimes referred to as Cdk11 (Tsutsui et al., 2008). The Cdk11 kinase associates with L-type cyclins (Dickinson et al., 2002; Loyer et al., 2008) and was implicated at several levels of transcription regulation (Hu et al., 2003; Trembley et al., 2002; Zong et al., 2005). Orthologous sequences to Cdk11 exist in all metazoans and fission yeast, but there is no budding yeast counterpart (Guo and Stiller, 2004; Liu and Kipreos, 2000).

RESULTS

A Cdk11-Cyclin L Complex Is Present in Fission Yeast and Affects Transcription Independently of CTD Phosphorylation

Genome sequence analyses revealed the existence of a sequence (*SPBC18H10.15*) encoding a protein related to the metazoan Cdk11 in fission yeast (Liu and Kipreos, 2000). We fused a green fluorescent protein (GFP) moiety to the fission yeast protein (hereafter named Cdk11) by gene targeting at the endogenous locus. Fluorescence microscopy revealed that Cdk11 was expressed and mainly concentrated in the nucleus (Figure 1A). Sequences alignment showed that the fission yeast Cdk11 was significantly shorter than its homologs, consisting only of the C-terminal kinase domain and an authentic PITSLRE signature (Figure S2A and S2B). Because metazoan Cdk11 exists in several isoforms, including a mitotic, truncated version (p58) corresponding to fission yeast Cdk11, we analyzed the expression level of Cdk11 during the *cdc25-22* block and release experiment, which did not highlight any cell cycle periodicity (Figure 1B). A tandem affinity purification (TAP) identified physical partners of Cdk11, including an uncharacterized cyclin (*SPAC1296.05c*) that was confirmed to bind Cdk11 in independent coimmunoprecipitation experiments (Figure 1C and 1D). Other interactors included transcription-associated proteins (Spt4 and Spt5), a Mediator subunit (Med10), RNA helicases (Moc2 and Dbp2), and some classical contaminants. None of these could be reproducibly coimmunoprecipitated in independent experiments. The cyclin, hereafter named L-type cyclin pombe (Lcp1), showed similarity to the L-type cyclin family previously shown to associate with metazoan Cdk11 (Dickinson et al., 2002; Loyer et al., 2008), but was significantly shorter than its homologs, due to the absence of the RS domain (Figure S2C and S2D). Taken together, these data support the existence in fission yeast of a divergent Cdk11-cyclin L complex that is absent from budding yeast. Deletions of either *cdk11* or *lcp1* were reported to be viable in large-scale studies (Bimbó et al., 2005; Kim et al., 2010), which we confirmed (data not shown).

The relationship of this complex to the CTD kinase family led us to analyze the effect of Cdk11 on CTD phosphorylation in vitro and in vivo. Precipitated Cdk11 or Lcp1 displayed very low kinase activity toward a fusion between glutathione S-transferase (GST) and the wild-type fission yeast CTD, in contrast to Mcs6 (the Cdk7 ortholog), which readily phosphorylated the GST-CTD fusion in vitro (Figure 1E) (Drogat and Hermand, 2012). In addition, while the inactivation of the well-described CTD serine 5 or serine 2 kinases (Mcs6 [Cdk7] and Lsk1 [Cdk12], respectively) specifically decreased the phosphorylation level of these two residues in vivo, the absence of *cdk11* had no effect (Figure 1F). Therefore, we found no evidence of Cdk11 being a genuine CTD kinase in fission yeast.

The Cdk11 and Mediator-Associated Cdk8 Kinase Regulate a Common Set of Genes

The previously reported connection between Cdk11 and transcription, together with its nuclear localization and copurification with transcription regulators (although at weak level) led us to test its chromatin association. Gene-specific chromatin immunoprecipitation (ChIP) experiments showed that Cdk11-hemagglutinin (HA) was enriched onto chromatin compared to an untagged control (data not shown), and a genome-wide ChIP-on-chip analysis showed a broad distribution of Cdk11-HA. Analysis of the average occupancy across genes showed a bimodal binding pattern of Cdk11 compared to the previously reported accumulation of PolII toward the 3′-end of the transcribed unit (Coudreuse et al., 2010) (Figure 2A). The Rpb3-HA and Cdk11-HA profiles at individual loci (see below) are shown in Figures 2B and S3A. The genome-wide distribution of Cdk11 contrasted with the absence of phenotype of the mutant, which suggests that, albeit Cdk11 associated with general transcription, it may only affect a subset of genes. This possibility was confirmed by global expression profiling, showing that only 55 genes were significantly affected in the absence of Cdk11. Interestingly, clustering analyses with the expression profiles of the related Cdk family member in fission yeast (Cdk7, Cdk8, Cdk9, and Cdk12 [Coudreuse et al., 2010], Figure S3B) showed that the absence of either *cdk11* or *cdk8* resulted in very similar defects (up- or downregulation) that were more pronounced in the *cdk8* mutant (Figure 2C). Quantitative RT-PCR confirmed this effect on representative genes and showed, in addition, that the expression defects were not cumulated in the double *cdk8 cdk11* mutant (Figure 2D). The analysis of an analog-sensitive (-as) mutant strongly supported that the kinase activity of Cdk11 was required in vivo (Figures 2D and S3D). Note that, in this study, we have chosen 10 representative genes that were the more strongly affected in the absence of Cdk11 for more detailed analysis. Their ChIP and expression profiles are shown in Figures 2B, S3A, and S3C.

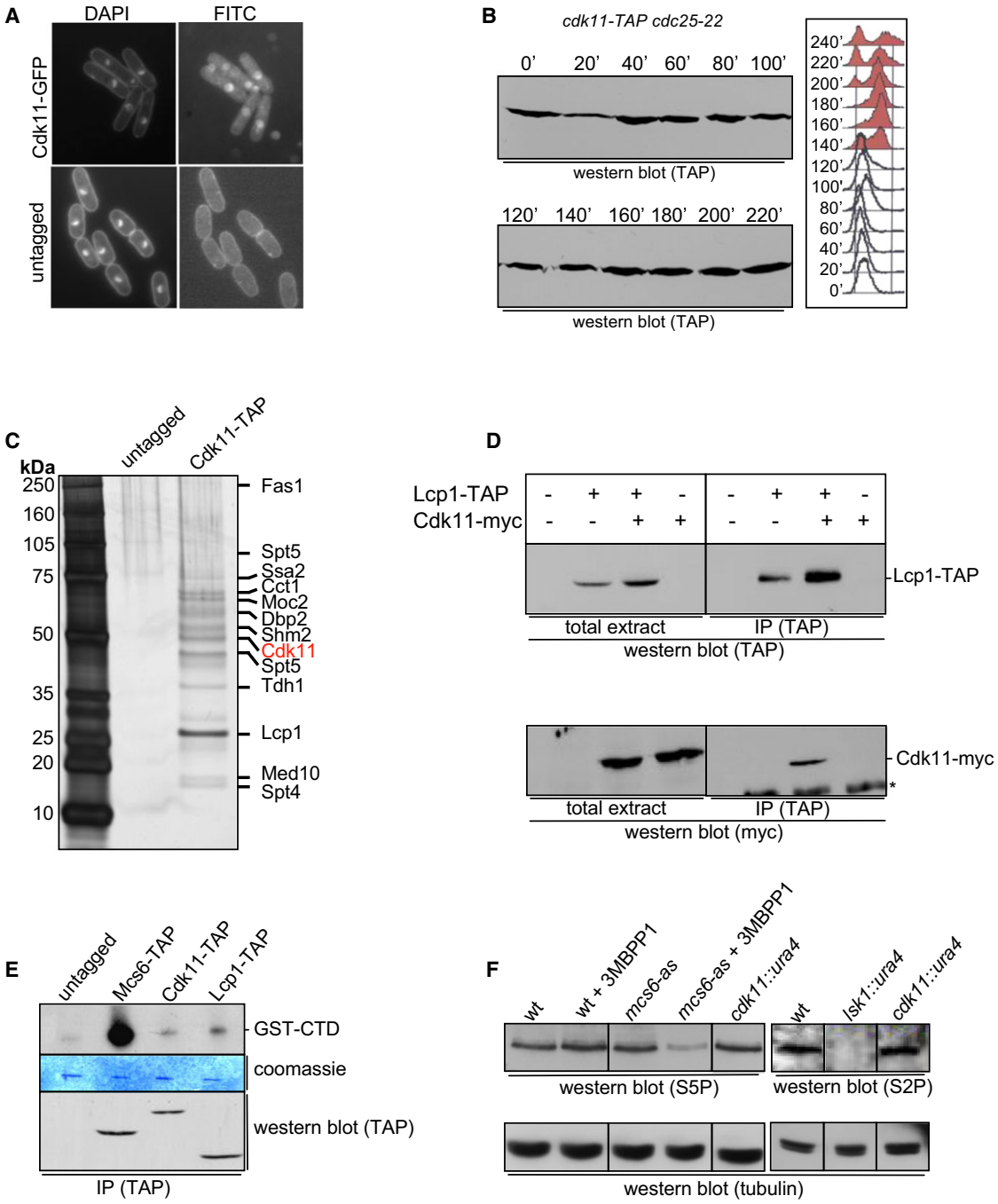

Figure 1. A Cdk11-Cyclin L Complex Is Present in Fission Yeast and Affects Transcription Independently of CTD Phosphorylation

(A) Cdk11-GFP expressed from the endogenous locus or an untagged control were observed by fluorescence microscopy in the presence of DAPI.

(B) The *cdk11-TAP* strain was synchronized using a *cdc25-22* block and release. At indicated times, samples were processed for TAP western blot and fluorescence-activated cell sorting analysis. The replicated population is marked in red.

(C) TAP of the *cdk11-TAP* and an untagged strain. Proteins identified from bands by mass spectrometry are indicated.

(D) Strains harboring the indicated tagged proteins were lysed and the Lcp1-TAP protein was precipitated on immunoglobulin G (IgG) beads. Total or precipitated products were resolved on PAGE and analyzed by western blot using the indicated antibodies. The asterisk indicates the IgG heavy chains.

(E) Strains harboring the indicated tagged proteins were lysed and immunoprecipitation was performed on IgG beads followed by kinase assay on GST-CTD. The kinase gel was stained with Coomassie blue. western blot analysis was performed using the peroxidase antiperoxidase antibody.

(F) The indicated strains were grown 2 hr in the presence or absence of the 3MB-PP1 inhibitor, lysed, and separated by PAGE. Western blot analyses were performed with the indicated antibodies.

See also Figure S2.

The Cdk11-Cyclin L Complex Phosphorylates Subunits of the Mediator Complex

Taken together, the previous data suggested a connection between the Cdk11-Lcp1 complex and the kinase submodule of the Mediator. We hypothesized that Cdk11 could phosphorylate some Mediator subunits to regulate the association of the Cdk8 kinase module. Indeed, global phosphoproteome analyses in fission yeast have identified phosphorylation on several Mediator subunits (Beltrao et al., 2009; Wilson-Grady et al., 2008). Using the integrated fission yeast ORFeome collection (Matsuyama et al., 2006), we set up a phosphorylation assay for all 23 Mediator subunits, allowing the precipitation of a tagged Mediator subunit from fission yeast extract and their use as a substrate for the Cdk11-Lcp1 kinase complex (Figure S4A). Six potential substrates (Med14, Med27, Med4, Med7, Med1, and Med19) were selected in this way and retested individually as GST-fusion proteins (Figure 3A). The experiments showed that Med4 and Med27 were readily phosphorylated by Cdk11 in vitro. Similarly to Cdk11, the Med27 protein is not conserved in budding yeast.

Combining the large-scale phosphoproteome data set and sequence alignment (Figures S4B and S4C), we determined that putative phosphoacceptors and in vitro analysis demonstrated that Cdk11 phosphorylates Med4 on three residues (Figure 3B: S115, S204, and S218), of which one is evolutionary conserved (S115), and Med27 on S235, which is also highly conserved (Figure 3C).

Phosphorylation of Med4 and Med27 by Cdk11-Lcp1 Regulates the Association of the Kinase Module with the S-Mediator

We next integrated and tagged the phosphorylation site mutants in *med4* and *med27* at their respective endogenous locus. Quantitative RT-PCR showed that, when combined, these mutants interfered with the expression of representative genes similarly to the *cdk8* or *cdk11* mutants (Figure 4A). Using the *med4* or *med27* mutants alone, we noticed a gene-specific sensitivity to these mutants, but the combined mutations generally showed synergism. These data were consistent with a regulation of Cdk8 binding to the S-Mediator by the Cdk11-Lcp1 complex. We tested this possibility by coimmunoprecipitation. We first analyzed the integrity of the S-Mediator by coprecipitating the middle subunit Med7 and the head subunit Med27 in the presence or absence of Cdk11. As shown in Figure 4B (left panel), no effect of Cdk11 could be highlighted. At the contrary, the interaction between the kinase module subunit Cdk8 and the head subunit Med27 was completely abrogated when Cdk11 was inactivated (Figure 4B, middle panel). This role of Cdk11 in Mediator integrity was likely mediated by phosphorylation of Med27 and Med4 on the sites identified above (Figures 3 and 4A), because the interaction between Cdk8 and either the Med27 or Med4 phosphorylation mutants was specifically lost (Figure 4B, right panel). In contrast, the phosphorylation mutants of Med27 and Med4 still interacted with the middle subunit Med7 (Figure 4B, right panel). These data indicate that the association of the kinase submodule and the S-Mediator requires the phosphorylation of Med27 and Med4 by Cdk11. To confirm these findings at the chromatin level, we performed ChIP analyses of Cdk8-TAP and Med13-TAP in the presence or absence of Cdk11. As predicted, the chromatin abundance of these two components of the kinase submodule was decreased in the absence of Cdk11 (Figure 4C, left and middle panels). In the same conditions, the chromatin occupancy of Med7 was not affected (Figure 4C, right panel).

DISCUSSION

The reversible association between the S-Mediator and the kinase module is pivotal in gene expression and decides if PolII is incorporated in active transcription or if its assembly is repressed (Taatjes, 2010). Here we have identified the fission yeast Cdk11-Lcp1 complex as a key regulator of that process through the phosphorylation of the Med27 and Med4 subunits. Similarly to Cdk8, Cdk11 affects transcription both positively and negatively (Figure 2), and additional work is needed to understand the molecular basis of the effect of the Cdk8 subcomplex on the RNA polymerase II.

Interestingly, neither Cdk11 nor Lcp1 or Med27 are conserved in budding yeast. Although it was proposed that Med3 is the ortholog of Med27 in that species (Bourbon, 2008), it lacks the conserved C-terminal zinc finger of Med27 and the highly conserved Cdk11 phosphorylation site. Contrary to the expectation based on the structure of budding yeast Mediator, fission yeast Med27 was found on the exterior of the complex, close to the head module (Linder et al., 2008). A similar case was reported for the mammalian Med29 subunit that physically binds the head (Sato et al., 2003a). These discrepancies between the overall Mediator structures may explain why the Cdk11-Lcp1 complex was lost in budding yeast (Liu and Kipreos, 2000). Contrary to Med27, the phosphorylation of Med4 exists in budding yeast and was suggested to modulate the assembly of the Mediator (Balciunas et al., 2003). It partially depends upon the Cdk7 ortholog (Guidi et al., 2004), which suggests a mutual targeting of Mediator and the Cdk7-containing complex transcription factor II H (TFIIH) (Esnault et al., 2008; Guidi et al., 2004).

Genome-wide studies in both yeasts revealed that Mediator and the kinase module are present upstream of protein coding genes, but also interact with the downstream coding region of many genes (Andrau et al., 2006; Zhu et al., 2006). The genome-wide occupancy of Cdk11 is consistent with these previous findings. Moreover, a transient association of the kinase module was suggested by the reduced occupancy of Cdk8 (Andrau et al., 2006), which highlights the reversible nature of the S-Mediator/kinase module interaction. How dynamic is the association at activated and repressed genes and how Cdk11 modulates this process requires further investigations.

The absence of two conserved domains in the fission yeast Cdk11 and cyclin orthologs and the reported essential roles of some Cdk11 isoforms in unrelated processes in metazoans (Petretti et al., 2006; Wilker et al., 2007; Yokoyama et al., 2008) raise the question of the conservation of the regulation described here. The well-established connection between Cdk11 and transcription together with the lethality of null

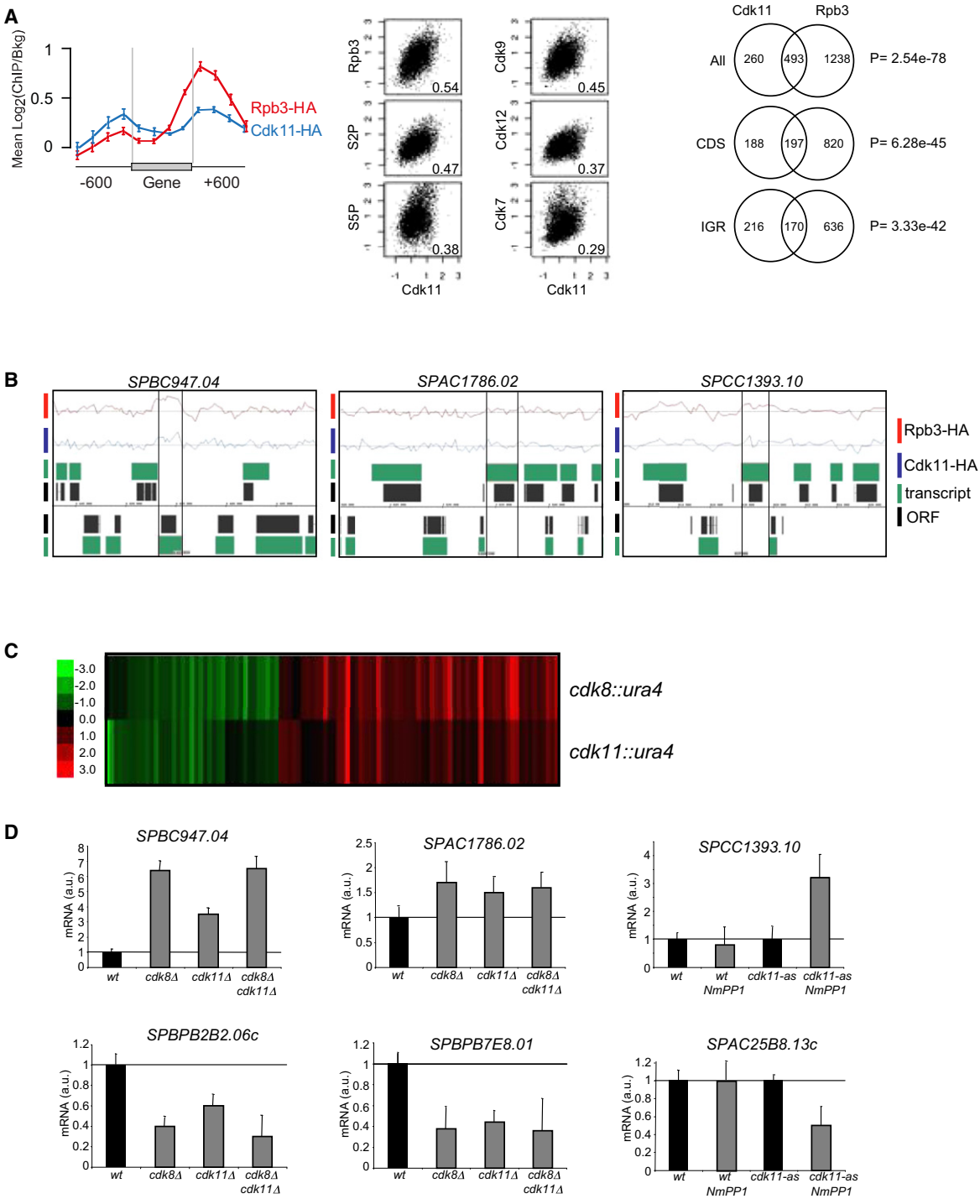

Figure 2. The Cdk11 and Mediator-Associated Cdk8 Kinase Regulate a Common Set of Genes

(A) Left: Average ChIP-binding profiles of genes that were significantly bound (binding ratio ≥2, and $p < 0.05$) by Cdk11-HA (blue) and Rpb3-HA (red). The 95% confidence intervals and a graphical representation of the transcribed units (Gene) are displayed. Middle: Plots showing the correlation between binding ratios of ChIP-on-chips experiments performed with the indicated proteins (based on previous data (Coudreuse et al., 2010), Cdk7 is SpMcs6 and Cdk12 is SpLsk1). The correlation coefficients for each pair-wise combination are indicated in the bottom right corner of the scatter plots. Right: Occupancy intersection of ChIP-binding peaks for the Cdk11/Rpb3 combination (ALL) and their repartition between coding regions (CDS) and intergenic regions (IGR) with associated p values obtained from hypergeometric tests.

(B) ChIP-on-chip occupancy profiles of Rpb3-HA and Cdk11-HA along the *SPBC947.04, SPAC1786.02*, and *SPCC1393.10* genomic regions. Transcripts and open reading frames (ORFs) are indicated.

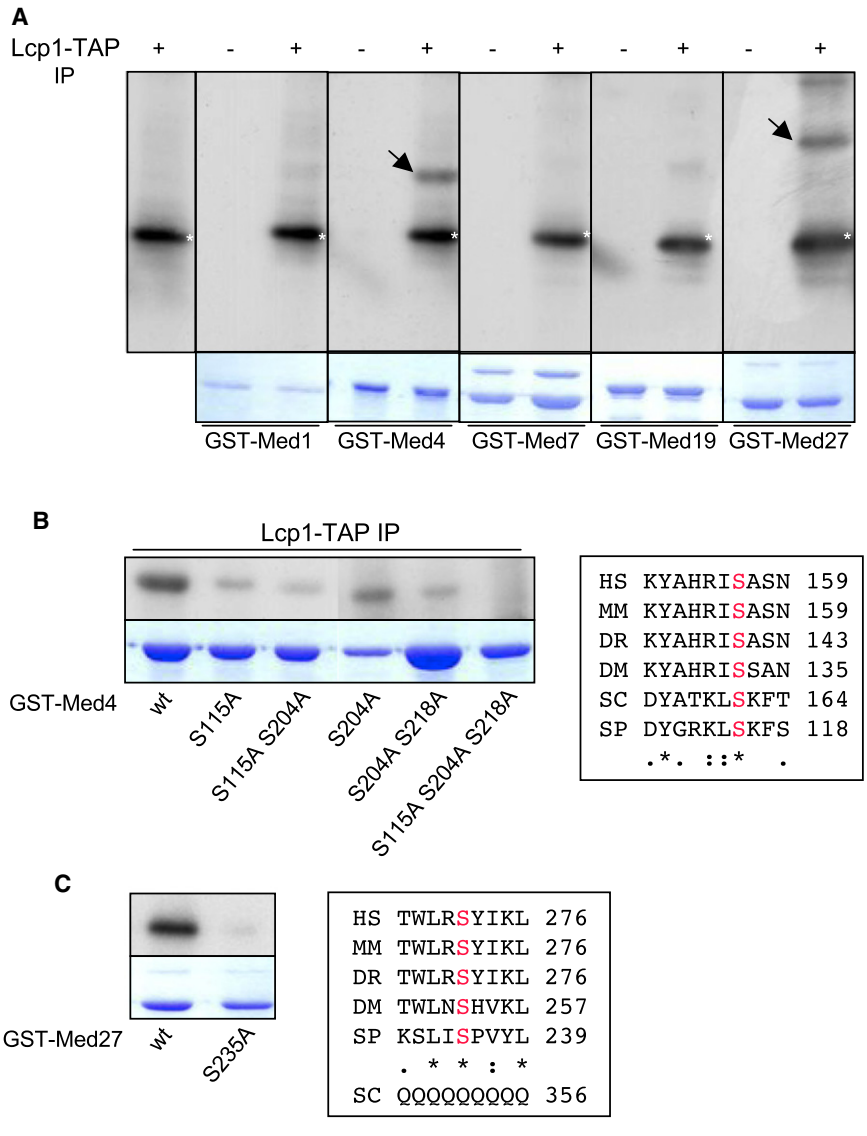

Figure 3. The Cdk11-Cyclin L Complex Phosphorylates Subunits of the Mediator Complex

(A) The Lcp1-TAP protein was precipitated and used on beads for in vitro kinase assay using various purified GST-fusion proteins as indicated. The amount of substrates was estimated by staining of the kinase gel.

(B) The Lcp1-TAP protein was precipitated and used on beads for in vitro kinase assay using various wild-type and mutated forms of the GST-Med4 protein as indicated. The conserved phosphorylation site (S115 in fission yeast) is shown on the right. The amount of substrates was estimated by staining of the kinase gel.

(C) The Lcp1-TAP protein was precipitated and used on beads for in vitro kinase assay using the wild-type and mutated forms of the GST-Med27 protein as indicated. The conserved phosphorylation site (S235 in fission yeast) is shown on the right. The amount of substrates was estimated by staining of the kinase gel. Note that this site is not conserved in budding yeast Med3, a putative ortholog of Med27.

See also Figure S4.

mutants of *Cdk8* or *Cdk11* at a similar stage in early embryogenesis (Li et al., 2004; Westerling et al., 2007) certainly supports this possibility.

EXPERIMENTAL PROCEDURES

General Methods

Fission yeast growth, microscopy, gene targeting, and mating were performed as described (Bamps et al., 2004; Cassart et al., 2012; Fersht et al., 2007; Hermand and Nurse, 2007). TAP was performed as described (Dewez et al., 2008; Guiguen et al., 2007). Kinase assay on GST-CTD were previously described (Coudreuse et al., 2010). Inhibitors of the analog-sensitive mutant kinase were obtained from Toronto Research Chemicals. The list of strains used in this study is shown in Table S1. The corrected cdk8 open reading frame, taking into account an intron located in the 3′ region, is shown in Figure S5.

ChIP-on-Chip, ChIP, and Quantitative RT-PCR

ChIP-on-chips were performed using the Agilent *S. pombe* 4 × 44 k microarrays (Whole Genome ChIP-on-chip Microarray [G4810A], design ID: 015424). The genome-wide binding profiles for Cdk11 were generated from four independent biological samples. Chromatin immunoprecipitations were performed using a Bioruptor (Diagenode) and Dynabeads (Invitrogen). Total RNA was prepared and purified on QIAGEN RNeasy. Quantitative RT-PCR was performed using the ABI high capacity RNA-to-complementary DNA (cDNA). The untreated sample was used as a reference and the *act1* mRNA was used for normalization (Bauer et al., 2012). More details are provided in the Supplemental Information.

Expression Profiling

Transcriptome analyses were performed on customized 4 × 44 k Agilent microarrays (Coudreuse et al., 2010). For each sample, 500 ng of total RNA was converted into labeled cDNA, with nucleotides coupled to a fluorescent dye (Cy3 or Cy5) using the Low RNA Input Linear Amplification Kit (Agilent Technologies). Two biological samples were hybridized for each mutant strain,

(C) Hierarchical clustering of the 90 mRNAs whose expression is significantly affected in the *cdk8::ura4* or the *cdk11::ura4* strains. Strains were grown in yeast extract supplemented (YES). The data are presented as a *log2* mutant/wild-type ratio of hybridization signals and are color-coded, as indicated in the key (p value $\leq$ 0.05 and $\geq$1.5-fold change).

(D) Relative quantification (RQ) of the indicated mRNAs determined by quantitative RT-PCR using the ΔΔct method in wild-type, *cdk8::ura4, cd11::ura4*, and the double *cdk8::ura4 cd11::ura4* mutants. a.u.: arbitrary units.

See also Figure S3.

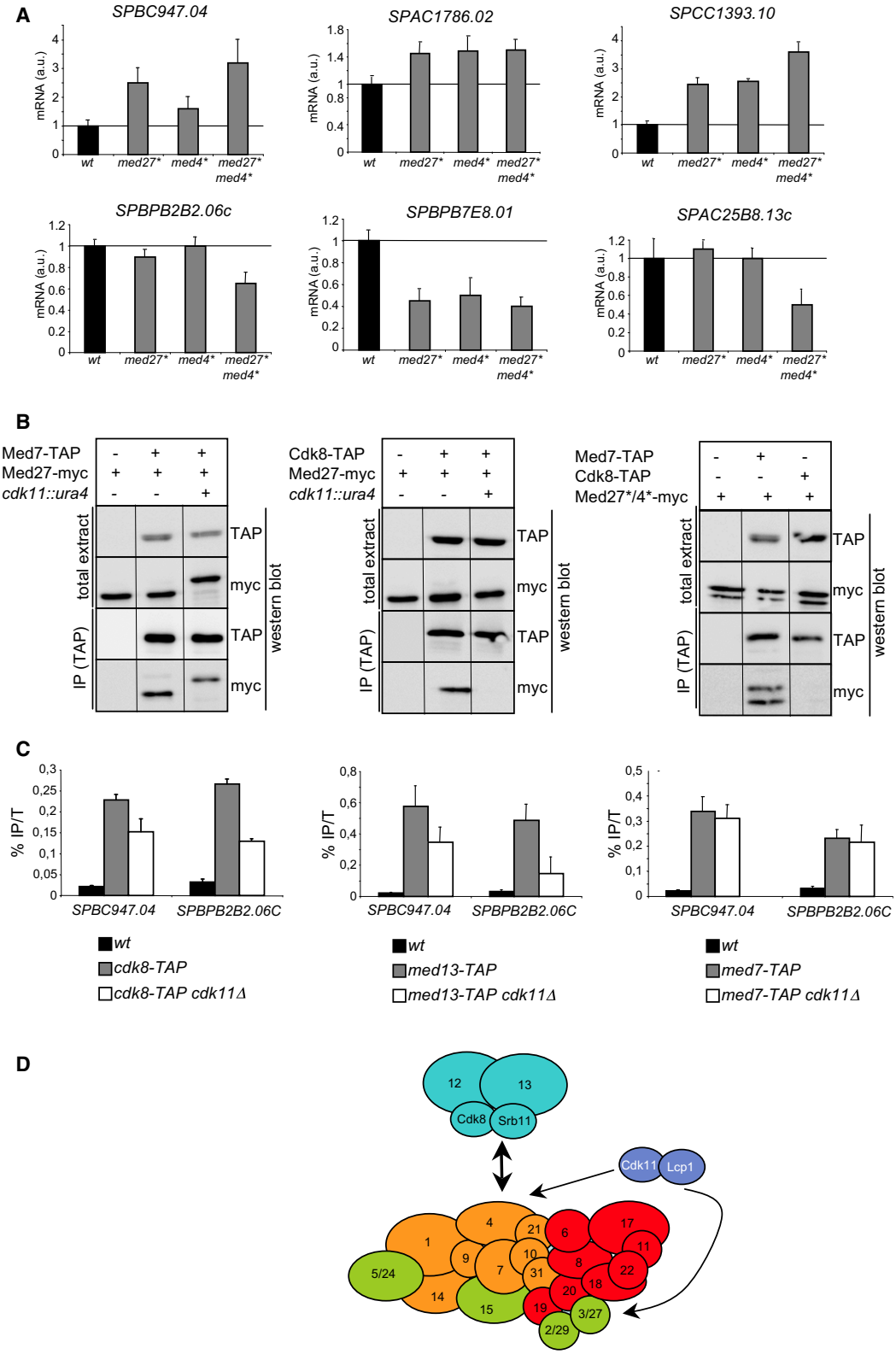

Figure 4. Phosphorylation of Med4 and Med27 by Cdk11-Lcp1 Regulates the Association of the Kinase Module with the S-Mediator

(A) RQ of the indicated mRNAs determined by quantitative RT-PCR using the ΔΔct method in wild-type, *med27 S235A (med27*), med4 S115A S204A S218A (med4*)*, and the double *med27* med4** mutants. a.u.: arbitrary units.

with two dye-swap technical replicates per sample. More details are provided in the Supplemental Information.

ACCESSION NUMBERS

The microarray data and the ChIP-on-chip data have been deposited at the Gene Expression Omnibus (NCBI-GEO) database under accession numbers GSE37960 and GSE37961.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, five figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2012.09.027.

LICENSING INFORMATION

ACKNOWLEDGMENTS

We thank Paul Nurse and Zsolt Szilagyi for reagents; Damien Coudreuse, Julie Soutourina, and Michel Werner for help with genome-wide analyses. We thank Tomi P Mäkelä and Jean-Christophe Andrau for critical reading of the manuscript and the GEMO laboratory for discussions. This work was supported by grants FRFC 2.4510.10, Credit aux chercheurs 1.5.013.09 and MIS F.4523.11 to D.H. D.H. is a FNRS Research Associate.

Received: June 11, 2012
Revised: August 30, 2012
Accepted: September 24, 2012
Published: November 1, 2012

REFERENCES

Andrau, J.C., van de Pasch, L., Lijnzaad, P., Bijma, T., Koerkamp, M.G., van de Peppel, J., Werner, M., and Holstege, F.C. (2006). Genome-wide location of the coactivator mediator: Binding without activation and transient Cdk8 interaction on DNA. Mol. Cell *22*, 179–192.

Asturias, F.J., Jiang, Y.W., Myers, L.C., Gustafsson, C.M., and Kornberg, R.D. (1999). Conserved structures of mediator and RNA polymerase II holoenzyme. Science *283*, 985–987.

Balciunas, D., Hallberg, M., Björklund, S., and Ronne, H. (2003). Functional interactions within yeast mediator and evidence of differential subunit modifications. J. Biol. Chem. *278*, 3831–3839.

Bamps, S., Westerling, T., Pihlak, A., Tafforeau, L., Vandenhaute, J., Mäkelä, T.P., and Hermand, D. (2004). Mcs2 and a novel CAK subunit Pmh1 associate with Skp1 in fission yeast. Biochem. Biophys. Res. Commun. *325*, 1424–1432.

Bauer, F., Matsuyama, A., Candiracci, J., Dieu, M., Scheliga, J.S., Wolf, D.A., Yoshida, M., and Hermand, D. (2012). Translational control of cell division by elongator. Cell Rep. *1*, 424–433.

Beltrao, P., Trinidad, J.C., Fiedler, D., Roguev, A., Lim, W.A., Shokat, K.M., Burlingame, A.L., and Krogan, N.J. (2009). Evolution of phosphoregulation: comparison of phosphorylation patterns across yeast species. PLoS Biol. *7*, e1000134.

Bimbó, A., Jia, Y., Poh, S.L., Karuturi, R.K., den Elzen, N., Peng, X., Zheng, L., O'Connell, M., Liu, E.T., Balasubramanian, M.K., and Liu, J. (2005). Systematic deletion analysis of fission yeast protein kinases. Eukaryot. Cell *4*, 799–813.

Bourbon, H.M. (2008). Comparative genomics supports a deep evolutionary origin for the large, four-module transcriptional mediator complex. Nucleic Acids Res. *36*, 3993–4008.

Cantin, G.T., Stevens, J.L., and Berk, A.J. (2003). Activation domain-mediator interactions promote transcription preinitiation complex assembly on promoter DNA. Proc. Natl. Acad. Sci. USA *100*, 12003–12008.

Cassart, C., Drogat, J., Migeot, V., and Hermand, D. (2012). Distinct requirement of RNA polymerase II CTD phosphorylations in budding and fission yeast. Transcription. Published online October 1, 2012. http://dx.doi.org/10.4161/trns.21066.

Conaway, R.C., and Conaway, J.W. (2011). Function and regulation of the Mediator complex. Curr. Opin. Genet. Dev. *21*, 225–230.

Cornelis, S., Bruynooghe, Y., Denecker, G., Van Huffel, S., Tinton, S., and Beyaert, R. (2000). Identification and characterization of a novel cell cycle-regulated internal ribosome entry site. Mol. Cell *5*, 597–605.

Coudreuse, D., van Bakel, H., Dewez, M., Soutourina, J., Parnell, T., Vandenhaute, J., Cairns, B., Werner, M., and Hermand, D. (2010). A gene-specific requirement of RNA polymerase II CTD phosphorylation for sexual differentiation in S. pombe. Curr. Biol. *20*, 1053–1064.

Dewez, M., Bauer, F., Dieu, M., Raes, M., Vandenhaute, J., and Hermand, D. (2008). The conserved Wobble uridine tRNA thiolase Ctu1-Ctu2 is required to maintain genome integrity. Proc. Natl. Acad. Sci. USA *105*, 5459–5464.

Dickinson, L.A., Edgar, A.J., Ehley, J., and Gottesfeld, J.M. (2002). Cyclin L is an RS domain protein involved in pre-mRNA splicing. J. Biol. Chem. *277*, 25465–25473.

Dotson, M.R., Yuan, C.X., Roeder, R.G., Myers, L.C., Gustafsson, C.M., Jiang, Y.W., Li, Y., Kornberg, R.D., and Asturias, F.J. (2000). Structural organization of yeast and mammalian mediator complexes. Proc. Natl. Acad. Sci. USA *97*, 14307–14310.

Drogat, J., and Hermand, D. (2012). Gene-specific requirement of RNA polymerase II CTD phosphorylation. Mol. Microbiol. *84*, 995–1004.

Elmlund, H., Baraznenok, V., Lindahl, M., Samuelsen, C.O., Koeck, P.J., Holmberg, S., Hebert, H., and Gustafsson, C.M. (2006). The cyclin-dependent kinase 8 module sterically blocks Mediator interactions with RNA polymerase II. Proc. Natl. Acad. Sci. USA *103*, 15788–15793.

Esnault, C., Ghavi-Helm, Y., Brun, S., Soutourina, J., Van Berkum, N., Boschiero, C., Holstege, F., and Werner, M. (2008). Mediator-dependent recruitment of TFIIH modules in preinitiation complex. Mol. Cell *31*, 337–346.

Fersht, N., Hermand, D., Hayles, J., and Nurse, P. (2007). Cdc18/CDC6 activates the Rad3-dependent checkpoint in the fission yeast. Nucleic Acids Res. *35*, 5323–5337.

Galbraith, M.D., Donner, A.J., and Espinosa, J.M. (2010). CDK8: a positive regulator of transcription. Transcription *1*, 4–12.

Garrett-Engele, C.M., Siegal, M.L., Manoli, D.S., Williams, B.C., Li, H., and Baker, B.S. (2002). intersex, a gene required for female sexual development in Drosophila, is expressed in both sexes and functions together with doublesex to regulate terminal differentiation. Development *129*, 4661–4675.

Guidi, B.W., Bjornsdottir, G., Hopkins, D.C., Lacomis, L., Erdjument-Bromage, H., Tempst, P., and Myers, L.C. (2004). Mutual targeting of mediator and the TFIIH kinase Kin28. J. Biol. Chem. *279*, 29114–29120.

(B) Strains harboring the indicated tagged proteins were lysed, and the TAP-tagged protein was precipitated on IgG beads. Total or precipitated products were resolved on PAGE and analyzed by western blot using the indicated antibodies. Note that both the Med27 S235A (Med27*) and the Med4 S115A S204A S218A (Med4*) mutants are myc-tagged. All relevant combinations were run on the same gel. Unprocessed blot images are presented in Figure S1A.
(C) The occupancy of Cdk8-TAP, Med13-TAP, and Med7-TAP was measured by ChIP at indicated loci in a wild-type strain and a *cdk11::ura4* mutant. The error bars were calculated from duplicates.
(D) A model of the association of the kinase module with the S-Mediator regulated by Cdk11-Lcp1 phosphorylation of Med27 and Med4.

Guiguen, A., Soutourina, J., Dewez, M., Tafforeau, L., Dieu, M., Raes, M., Vandenhaute, J., Werner, M., and Hermand, D. (2007). Recruitment of P-TEFb (Cdk9-Pch1) to chromatin by the cap-methyl transferase Pcm1 in fission yeast. EMBO J. *26*, 1552–1559.

Guo, Z., and Stiller, J.W. (2004). Comparative genomics of cyclin-dependent kinases suggest co-evolution of the RNAP II C-terminal domain and CTD-directed CDKs. BMC Genomics *5*, 69.

Hermand, D., and Nurse, P. (2007). Cdc18 enforces long-term maintenance of the S phase checkpoint by anchoring the Rad3-Rad26 complex to chromatin. Mol. Cell *26*, 553–563.

Holstege, F.C., Jennings, E.G., Wyrick, J.J., Lee, T.I., Hengartner, C.J., Green, M.R., Golub, T.R., Lander, E.S., and Young, R.A. (1998). Dissecting the regulatory circuitry of a eukaryotic genome. Cell *95*, 717–728.

Hu, D., Mayeda, A., Trembley, J.H., Lahti, J.M., and Kidd, V.J. (2003). CDK11 complexes promote pre-mRNA splicing. J. Biol. Chem. *278*, 8623–8629.

Kelleher, R.J., 3rd, Flanagan, P.M., and Kornberg, R.D. (1990). A novel mediator between activator proteins and the RNA polymerase II transcription apparatus. Cell *61*, 1209–1215.

Kim, D.U., Hayles, J., Kim, D., Wood, V., Park, H.O., Won, M., Yoo, H.S., Duhig, T., Nam, M., Palmer, G., et al. (2010). Analysis of a genome-wide set of gene deletions in the fission yeast Schizosaccharomyces pombe. Nat. Biotechnol. *28*, 617–623.

Knuesel, M.T., Meyer, K.D., Bernecky, C., and Taatjes, D.J. (2009). The human CDK8 subcomplex is a molecular switch that controls Mediator coactivator function. Genes Dev. *23*, 439–451.

Li, T., Inoue, A., Lahti, J.M., and Kidd, V.J. (2004). Failure to proliferate and mitotic arrest of CDK11(p110/p58)-null mutant mice at the blastocyst stage of embryonic cell development. Mol. Cell. Biol. *24*, 3188–3197.

Linder, T., Rasmussen, N.N., Samuelsen, C.O., Chatzidaki, E., Baraznenok, V., Beve, J., Henriksen, P., Gustafsson, C.M., and Holmberg, S. (2008). Two conserved modules of Schizosaccharomyces pombe Mediator regulate distinct cellular pathways. Nucleic Acids Res. *36*, 2489–2504.

Liu, J., and Kipreos, E.T. (2000). Evolution of cyclin-dependent kinases (CDKs) and CDK-activating kinases (CAKs): differential conservation of CAKs in yeast and metazoa. Mol. Biol. Evol. *17*, 1061–1074.

Loyer, P., Trembley, J.H., Grenet, J.A., Busson, A., Corlu, A., Zhao, W., Kocak, M., Kidd, V.J., and Lahti, J.M. (2008). Characterization of cyclin L1 and L2 interactions with CDK11 and splicing factors: influence of cyclin L isoforms on splice site selection. J. Biol. Chem. *283*, 7721–7732.

Matsuyama, A., Arai, R., Yashiroda, Y., Shirai, A., Kamata, A., Sekido, S., Kobayashi, Y., Hashimoto, A., Hamamoto, M., Hiraoka, Y., et al. (2006). ORFeome cloning and global analysis of protein localization in the fission yeast Schizosaccharomyces pombe. Nat. Biotechnol. *24*, 841–847.

Myers, L.C., Gustafsson, C.M., Bushnell, D.A., Lui, M., Erdjument-Bromage, H., Tempst, P., and Kornberg, R.D. (1998). The Med proteins of yeast and their function through the RNA polymerase II carboxy-terminal domain. Genes Dev. *12*, 45–54.

Myers, L.C., Gustafsson, C.M., Hayashibara, K.C., Brown, P.O., and Kornberg, R.D. (1999). Mediator protein mutations that selectively abolish activated transcription. Proc. Natl. Acad. Sci. USA *96*, 67–72.

Nonet, M.L., and Young, R.A. (1989). Intragenic and extragenic suppressors of mutations in the heptapeptide repeat domain of Saccharomyces cerevisiae RNA polymerase II. Genetics *123*, 715–724.

Petretti, C., Savoian, M., Montembault, E., Glover, D.M., Prigent, C., and Giet, R. (2006). The PITSLRE/CDK11p58 protein kinase promotes centrosome maturation and bipolar spindle formation. EMBO Rep. *7*, 418–424.

Samuelsen, C.O., Baraznenok, V., Khorosjutina, O., Spahr, H., Kieselbach, T., Holmberg, S., and Gustafsson, C.M. (2003). TRAP230/ARC240 and TRAP240/ARC250 Mediator subunits are functionally conserved through evolution. Proc. Natl. Acad. Sci. USA *100*, 6422–6427.

Sato, S., Tomomori-Sato, C., Banks, C.A., Parmely, T.J., Sorokina, I., Brower, C.S., Conaway, R.C., and Conaway, J.W. (2003a). A mammalian homolog of Drosophila melanogaster transcriptional coactivator intersex is a subunit of the mammalian Mediator complex. J. Biol. Chem. *278*, 49671–49674.

Sato, S., Tomomori-Sato, C., Banks, C.A., Sorokina, I., Parmely, T.J., Kong, S.E., Jin, J., Cai, Y., Lane, W.S., Brower, C.S., et al. (2003b). Identification of mammalian Mediator subunits with similarities to yeast Mediator subunits Srb5, Srb6, Med11, and Rox3. J. Biol. Chem. *278*, 15123–15127.

Spahr, H., Khorosjutina, O., Baraznenok, V., Linder, T., Samuelsen, C.O., Hermand, D., Mäkela, T.P., Holmberg, S., and Gustafsson, C.M. (2003). Mediator influences Schizosaccharomyces pombe RNA polymerase II-dependent transcription in vitro. J. Biol. Chem. *278*, 51301–51306.

Taatjes, D.J. (2010). The human Mediator complex: a versatile, genome-wide regulator of transcription. Trends Biochem. Sci. *35*, 315–322.

Thompson, C.M., and Young, R.A. (1995). General requirement for RNA polymerase II holoenzymes in vivo. Proc. Natl. Acad. Sci. USA *92*, 4587–4590.

Trembley, J.H., Hu, D., Hsu, L.C., Yeung, C.Y., Slaughter, C., Lahti, J.M., and Kidd, V.J. (2002). PITSLRE p110 protein kinases associate with transcription complexes and affect their activity. J. Biol. Chem. *277*, 2589–2596.

Tsutsui, T., Umemura, H., Tanaka, A., Mizuki, F., Hirose, Y., and Ohkuma, Y. (2008). Human mediator kinase subunit CDK11 plays a negative role in viral activator VP16-dependent transcriptional regulation. Genes Cells *13*, 817–826.

Westerling, T., Kuuluvainen, E., and Mäkelä, T.P. (2007). Cdk8 is essential for preimplantation mouse development. Mol. Cell. Biol. *27*, 6177–6182.

Wilker, E.W., van Vugt, M.A., Artim, S.A., Huang, P.H., Petersen, C.P., Reinhardt, H.C., Feng, Y., Sharp, P.A., Sonenberg, N., White, F.M., and Yaffe, M.B. (2007). 14-3-3sigma controls mitotic translation to facilitate cytokinesis. Nature *446*, 329–332.

Wilson-Grady, J.T., Villén, J., and Gygi, S.P. (2008). Phosphoproteome analysis of fission yeast. J. Proteome Res. *7*, 1088–1097.

Yokoyama, H., Gruss, O.J., Rybina, S., Caudron, M., Schelder, M., Wilm, M., Mattaj, I.W., and Karsenti, E. (2008). Cdk11 is a RanGTP-dependent microtubule stabilization factor that regulates spindle assembly rate. J. Cell Biol. *180*, 867–875.

Zhu, X., Wirén, M., Sinha, I., Rasmussen, N.N., Linder, T., Holmberg, S., Ekwall, K., and Gustafsson, C.M. (2006). Genome-wide occupancy profile of mediator and the Srb8-11 module reveals interactions with coding regions. Mol. Cell *22*, 169–178.

Zong, H., Li, Z., Liu, L., Hong, Y., Yun, X., Jiang, J., Chi, Y., Wang, H., Shen, X., Hu, Y., et al. (2005). Cyclin-dependent kinase 11(p58) interacts with HBO1 and enhances its histone acetyltransferase activity. FEBS Lett. *579*, 3579–3588.