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Cdc14 phosphatase promotes segregation of telomeres through repression of RNA polymerase II transcription

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Abstract

Kinases and phosphatases regulate mRNA synthesis through post-translational modification of the C-terminal domain (CTD) of the largest subunit of RNA polymerase II [1]. In yeast, the phosphatase Cdc14 is required for mitotic exit [2,3] and for segregation of repetitive regions [4]. Cdc14 is also a subunit of the silencing complex RENT [5,6], but no roles in transcription repression have been described. Here we report that inactivation of Cdc14 causes silencing defects at the intergenic spacer sequences (IGS) of ribosomal genes during interphase and at Y' repeats in sub-telomeric regions during mitosis. We show that Cdc14 role in silencing is independent from the RENT deacetylase subunit Sir2. Instead, Cdc14 acts directly on RNA Polymerase II by targeting CTD phosphorylation at S_2 and S_5. We also find that Cdc14 role as a CTD phosphatase is conserved in humans. Finally, telomere segregation defects in *cdc14* mutants [4] correlate with the presence of sub-telomeric Y' elements and can be rescued by transcriptional inhibition of RNA Pol II.

The conserved Cdc14 (cell division cycle 14)-family of phosphatases regulates several key events during late mitosis, most notably they promote reversal of Cdk1-dependent phosphorylation and thus mitotic exit [2,3,5]. In budding yeast, Cdc14 localises dynamically to different cellular structures in a cell-cycle dependent manner. During interphase Cdc14 is bound to the nucleolus [6] while at anaphase it is released throughout the cell [7]. Two regulatory networks activate Cdc14 during mitosis, FEAR (Cdc Fourteen Early Anaphase Release) and MEN (Mitotic Exit Network) [3]. The FEAR activation occurs in early anaphase and is important to coordinate many anaphase events, while MEN operates in late

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anaphase [3]. Cdc14 activation by FEAR is crucial for the faithful execution of many anaphase processes, including timely chromosome segregation. This role was identified through the observation that segregation errors are present in Cdc14 mutants but not MEN mutants [4,8,9]. Interestingly, such defects occur at specific genome regions, namely the repetitive ribosomal gene array (rDNA) and telomeres [4,8,9]. The failure to segregate ribosomal repeats in *cdc14* mutants is caused by lack of RNAP-I transcription inhibition [10-12], a process required for the loading of Condensin complex to these repeats during anaphase. In contrast, the role of Cdc14 in telomere segregation is unknown. Importantly, Cdc14 inactivation in cells tricked to transcribe ribosomal genes with RNAP-II, instead of RNAP-I, also causes segregation failure of the ribosomal repeats [11]. Therefore, one hypothesis would be that Cdc14 promotes transcription repression of RNAP-II genes at sub-telomeric sites. This possibility is particularly appealing because nucleolar Cdc14 was originally discovered as a subunit of the silencing RENT (regulator of nucleolar silencing and telophase exit) complex [5,6], which inhibits transcription by RNAP-II at the rDNA intergenic spacers (IGS). Besides Cdc14, RENT contains Sir2 and Cfi1/Net1 [5,6]. Roles for Sir2 and Net1 in rDNA silencing have been described extensively [13-15], however a role for Cdc14 in RNAP-II silencing has not been carefully assessed.

Growth assays are available for the examination of transcription silencing within the silenced loci in the yeast genome, however the contribution of essential genes, like Cdc14, to silencing cannot be analysed using these methods. To circumvent this limitation, we used RT-qPCR to measure levels of endogenous transcripts of the intergenic sequence (IGS) regions within yeast ribosomal repeats on chromosome XII (Fig. 1a) in the conditional mutant *cdc14-1* at permissive (25°C) and non-permissive (37°C) temperatures. An increase in IGS transcripts was observed in *cdc14-1* mutants at 37°C (relative to 25°C), but not in wild type cells (Fig. 1b-c). To test whether Cdc14 effect on IGS transcription is independent from its role in mitotic exit during anaphase, we measured total IGS transcription in *cdc14-1* and wild type cells blocked in G1 and G2/M at 37°C. An increase in IGS transcripts, particularly at IGS2, was observed for *cdc14-1* cells in these arrests (Fig. 1d-e), therefore Cdc14 plays a role in rDNA silencing independent of its main cell cycle function during anaphase.

In *cdc14-1* mutants cells, exit from mitosis is prevented under non-permissive conditions leading to cell cycle arrest in telophase. To ensure that the increased IGS transcription observed (Fig. 1b-d) is not due to the cell cycle arrest, we compared *cdc14-1* to *cdc15-2* mutants, which also arrest in telophase. Importantly, Cdc14 is bound to IGS regions in the final telophase arrest by *cdc15-2* inactivation [7], which provides an ideal control for a telophase arrest with Cdc14 bound to IGS regions. An similar increase in IGS transcripts was observed in the *cdc14-1* anaphase arrest relative to *cdc15-2* (Fig. 1f). These results confirm that, like other RENT members, Cdc14 contributes to rDNA silencing.

Sir2 is an NAD-dependent deacetylase that targets histone H3 and H4 to promote rDNA silencing [16]. It is possible that the role of Cdc14 in rDNA silencing (Fig. 1b-e) is fully mediated by Sir2 function. To test this, we first investigated whether Sir2 localises to IGS regions in the absence of Cdc14. We found that Cdc14 inactivation reduced Sir2 binding across IGS (Fig. 2a). Next, we investigated whether Cdc14 inactivation affected rDNA silencing in the absence of Sir2. We found increased IGS transcription in double mutant cells (*sir2*Δ *cdc14-1*) compared to single mutants (Fig. 2b), demonstrating that in the absence of Sir2, Cdc14 supports silencing at IGS regions.

In addition to silencing, transcription of non-coding RNA within the IGS regions is also subject to degradation, a process mediated by the TRAMP complex and by the exosome [17]. Next we investigated whether increased IGS transcription in *cdc14-1* cells is caused by

defects of this degradation pathway. To this aim, we deleted Trf4, the poly(A) polymerase component of TRAMP4, in *cdc14-1* cells and investigated IGS transcription in the presence and absence of Cdc14 (Fig. 2c). Double mutant *trf4*Δ *cdc14-1* cells showed an increase in IGS transcription compared to single mutants (Fig. 2c), which demonstrates that elevated IGS transcription in *cdc14-1* cells is not caused by defects in TRAMP-mediated degradation of ncRNA.

The transcription activity of RNAP-II is regulated through phosphorylation of the carboxyl-terminal domain (CTD) of the core subunit Rpb1 [18]. CTD contains tandem repeats of the heptapeptide, Tyr-Ser-Pro-Thr-Ser-Pro-Ser ($Y_1S_2P_3T_4S_5P_6S_7$) [18]. RNAP-II recruitment to promoters requires CTD to be hypo-phosphorylated, however transcription initiation involves phosphorylation on the serine-5 residues (S_5P) of CTD, and elongation on serine-2 (S_2P) [18]. Although RNAP-II has been shown to bind to IGS regions, it does not elongate [19], and thus the regions are silenced [13-15].

Since Cdc14 is a proline-directed phosphatase and both S_2 and S_5 residues on the CTD are followed by proline, we explored whether these CTD residues are a substrate of Cdc14. We incubated purified Cdc14 (GST-Cdc14), expressed in bacterial cells, with Rpb1 extracted from yeast. In the absence of Cdc14, Rpb1 phosphorylation was detected on both S_2 and S_5 (Fig. 2d). Phosphorylation at both S_2 and S_5 was rapidly and completely lost after incubation with Cdc14 (Fig. 2d). To confirm Cdc14 specificity in the *in vitro* phosphatase assay, several controls were also performed. Rpb1 incubation with a phosphatase-inactive Cdc14 (GST-Cdc14CR) [20], the PP2A catalytic subunit Pph21 [21] or the dual-specificity protein phosphatase (DSP) Msg5 [22] did not affect S_5 or S_2 phosphorylation (Fig. 2d). In contrast, incubation with the CTD S_5 phosphatase Ssu72 [23] led to loss of S_5 but not S_2 phosphorylation (Fig. 2d). *In vivo*, Cdc14 activity as a CTD phosphatase was consistent with an observed increase in CTD S_5 and S_2 phosphorylation on the IGS region upon Cdc14 inactivation (Fig. 2e-f).

Our findings demonstrate that Cdc14 binding to IGS regions in interphase contributes to their silencing (Fig. 1). Although Cdc14 is confined to rDNA during interphase, it is released from the nucleolus during anaphase through the sequential activation of the FEAR (Cdc Fourteen Early Anaphase Release) and MEN (Mitotic Exit Network) regulatory networks [3]. Next, we sought to determine whether the release of Cdc14 during anaphase affects transcription of RNA Pol-II genes outside rDNA. To investigate this, we compared transcription profiles on genome-wide strand-specific tiling arrays [24] in *cdc14-1* and *cdc15-2* arrested cells. Comparison of *cdc14-1* and *cdc15-2* transcription in the arrays across the IGS regions confirmed the increase in IGS transcription previously observed for *cdc14-1* (Supl. Fig. 1). Next, we looked for regions that showed an increase in transcription in *cdc14-1* relative to *cdc15-2*. We found no significant changes in the profiles of *cdc14-1* and *cdc15-2* arrests genome-wide (data not shown), with the exception of sub-telomeric regions that contained Y' elements. Strikingly, all telomeres containing Y' elements showed an increase in transcription in *cdc14-1* relative to *cdc15-2* (Fig. 3a-b and Supl. Fig. 2-3). In contrast, transcription profiles of telomeres lacking Y' elements were identical in *cdc14-1* and *cdc15-2* (Fig. 3c). We observed these Cdc14-dependent transcription changes in Y' elements to occur specifically during anaphase (Fig. 3d-e). Supporting this data, we found that Cdc14 is enriched on the Y' element of the right arm of chromosome IV (*TEL4R-Y'*) (Fig. 3f) and that CTD phosphoryation increased in the absence of Cdc14 activity in this region (Fig. 3g-h).

In budding yeast, Cdc14 is required for the segregation of repetitive regions, mainly the ribosomal gene array (rDNA) [4,8,9] and telomeres [4]. The rDNA non-disjunction phenotype observed in *cdc14-1* cells stems from a defect in repressing transcription of ribosomal

genes [10-12], which is a requirement for Condensin recruitment to rDNA [4,25] and anaphase disjunction of the locus [26]. Next, we sought to determine whether Condensin binds to the sub-telomeric Y' element on *TEL4R* in a Cdc14-dependent manner during anaphase. To this aim, we compared binding of Condensin subunit Smc2 in *cdc14-1* on the rDNA IGS and *TEL4R-Y'* regions (Fig. 4a-b). Consistent with previous reports, we observed that Condensin binding to rDNA is reduced in the absence of Cdc14 [4,25] (Fig. 4a). We note that the Smc2 binding profile in G1 arrested cells showed accumulation on *IGS1* but not *IGS2* (Fig. 4b). We presently do not know the exact reason for this, however, Condensin has been recently shown to form a ring that topologically entraps DNA [27], therefore it is possible that like in the case of Cohesin [28], the transcription machinery can slide Condensin through the template. Next, we investigated the effect of Cdc14 on Condensin binding to telomeric regions during anaphase. We found that while Smc2 binds to both telomeric regions of chromosome IV (*TEL4L*, lacking Y' elements, and *TEL4R-Y'*) in *cdc15-2* arrests (Fig. 4c), it binds to *TEL4L* but not *TEL4R-Y'* in cdc14-1 arrested cells (Fig. 4c). This result demonstrates that Smc2 binds to sub-telomeric Y' elements in a Cdc14-dependent manner during anaphase. Unlike Condensin, Sir2 binding on *TEL4R-Y'* was unaffected by Cdc14 inactivation (Fig. 4d). Therefore the Cdc14 function at telomeric Y' elements is independent of Sir2. Recent reports demonstrated that the related rDNA structural complex Cohibin has a Sir2-dependent role telomere silencing and stability [29]. This may point to division of labour in genome maintenance between closely related complexes.

The lack of Condensin binding to *TEL4R-Y'* but not *TEL4L* in *cdc14-1* arrests prompted us to investigate the segregation of chromosome IV in these arrests. We introduced arrays of *tet* operators (tetO) at different positions on this chromosome; at centromere proximal regions (*CEN4*-dot; 150kb away from *CEN4*), the left telomere (*TEL4L*-dot; 30kb away from *TEL4L*), the right telomere (*TEL4R*-dot; 30kb away from *TEL4R*) and two interstitial sites on the right arm (*ARM1*-dot and *ARM2*-dot) (Fig. 5a). We found that *TEL4L*-dots and *CEN4*-dots were fully segregated in the *cdc14-1* arrest while *TEL4R*-dots did not separate despite evident segregation of nuclear masses (Fig. 5a-b). An intermediate degree of segregation was observed for the *ARM*-dots (Fig. 5a). We note that the *TEL4R*-dot segregation failure was characterised by the presence of a single GFP dot in one of the segregated nuclei (Fig. 5b). There are two potential interpretations for this phenotype. One possibility is that replication of telomeric regions containing Y' elements is incomplete, since Cdc14 has been recently implicated in replication termination at the rDNA [30]. The second possibility, and more likely, is that lack of Condensin loading at these regions (Fig. 4b) prevents resolution of intertwines between sister chromatids [31,32].

To test whether the telomeric segregation defects in *cdc14-1* cells was caused by transcription of Y' elements, we used α-amanitin, a RNAP-II specific inhibitor [33], to block RNAP-II transcription. Addition of the drug improved significantly the degree of separation of the *ARM* and *TEL4R*-dots (Fig. 5a). Therefore, telomere segregation defects in *cdc14-1* mutants correlate with the presence of Y' elements in sub-telomeric regions and active RNAP-II transcription.

Cdc14 is a highly conserved phosphatase. In the human genome two Cdc14 homologues have been identified, hCDC14A and hCDC14B 14. CDC14A brings about mitotic CDK inactivation [34], and its depletion by RNAi leads to centrosome duplication, mitotic and cytokinesis defects [35], suggesting a role in chromosome partitioning and genome stability. It is differentially expressed in human cancer cells, interacts with Cdk1/cyclin B complex and p53 [36], and exhibits cytoplasmic localization during interphase [37]. In contrast, CDC14B, like yeast Cdc14, localizes to the nucleolus in interphase but not during mitosis [37]. Recent studies have linked CDC14B to the DNA damage checkpoint response during the G2 stage of the cell cycle [38]. The role of Cdc14 as an RNAP-II phosphatase in yeast prompted us to

investigate whether this is an evolutionarily conserved function. To this end, we expressed and purified the human Cdc14 homologue hCDC14A from bacterial cells and tested its phosphatase activity *in vitro* on human hRPB1 purified from HEK293T cells. Incubation of hRPB1 with purified hCDC14A led to dissapearance of S_2 and S_5 phosphorylation (Fig. 5c).

In summary, our study identifies a novel and conserved role for the mitotic exit phosphatase Cdc14 in the repression of RNA polymerase II transcription in repetitive regions of the yeast genome. Moreover, Cdc14 function links RNAP-II and chromosome segregation through the evidence that transcriptional repression of sub-telomeric regions containing Y' elements by Cdc14 facilitates Condensin loading to and segregation of these telomeres. We propose that mitotic RNAP-II transcription inhibition facilitates loading of factors that mediate structural functions on mitotic chromosomes.

Methods

Strains and materials

All relevant yeast strains used are shown in table S1. C-terminal epitope tagging of proteins was performed using PCR allele replacement methods. Anti-Myc 9E10 monoclonal antibody and anti-HA 12CA5 monoclonal antibody used (Roche). Cells were harvested from exponentially growing cultures at different temperatures or different sugar sources, depending of the strains used, and stored at −80°C. The GST-Cdc14 and GST-Cdc14 phosphatase-dead mutant (*CDC14-C283S/R289*; Cdc14-C/S), as well as yeast strains expressing Cdc14 from the *GAL1-10* promoter were gifts from A. Amon and D. Morgan. Constructs to target 5.6 kb of tetO arrays to different sites on the long arm of chromosome IV were generated by gene synthesis. Chromosome tags were inserted adjacent to *COS7* (*TEL4L*-dot), *RAD55* (*CEN4*-dot), *ARS430* (*ARM1*-dot), *PRP3* (*ARM2*-dot) and *IRC4* (*TEL4R*-dot) loci. Details are available on request. Tag integrations were confirmed by PCR.

Cell cycle experiments

Cells arrested in G1 were treated with α-factor. To release cells from α-factor block, we transferred them to fresh media plus pronase E (0.1 mg/ml). For releases at non-permissive temperatures we exposed cells to 37°C for 30min before transfer to fresh media (also at 37°C). Metaphase arrests using nocodazole were done incubating cells for 3 hours with 15μg/ml nocodazole. Growth, arrest and release conditions and concentration of drugs used for every experiment shown are explained in figure legends. For segregation experiments, *cdc14-1* cells were grown at 25°C in 2% glucose before shifting the temperature to 37°C for 2 hours to inactivate Cdc14. *cdc14-1* cells arrested in telophase were treated with 66μg/ml of α-amanitin or kept untreated for 2 hours.

Cell extracts and western blotting

To make protein extracts, cell cultures were centrifuged and resuspended in 20μl of RIPA buffer (10mM Sodium Phosphate, 1% Triton X-100, 0.1% SDS, 10mM EDTA, 150mM NaCl, pH7). Cells were physically broken using glass beads in a Fast Prep disruptor machine (2 pulses, 20 seconds each). The total cell extract was recovered by washing the beads with 200μl of RIPA buffer. The soluble proteins were obtained by centrifugation for 5 minutes at 12.000 rpm at 4°C. Protein concentrations were measured using BCA Protein Assay (Sigma). For SDS gels, 30μg of total cell extract was loaded in 8% polyacrylamide gels in Tris-Glicine buffer (Tris 3g/l, Glicine 14.4 g/l, SDS 1g/l). Following gel runs at 30mA, proteins were transferred to Hybond-P membranes (Amersham-Bioscience) in Tris-Glicine buffer without SDS. Membranes were blocked with 5% milk/BSA in PBS, 0.1% Tween®-20 (Sigma). Addition of the primary antibodies was as follows: phosphoSer2 was

used at a concentration of 1:1000 (provided by D. Eick), BSA was used as a blocking agent instead of milk, H5 antiphosphoS2 (Abcam-24758) was used at a concentration of 1:5000, phosphoSer5 antibody was used at a concentration of 1:5000, using milk as a blocking agent, (Abcam-5408). Following primary antibody incubation, membranes were washed (1×15minutes and 2×5 minutes) with PBS-Tween. Secondary antibodies were added at a 1:20.000 concentration. Following incubation with secondary antibody membranes were washed (1×20 minutes, 2×5 minutes) with PBS-Tween and (2×10 minutes) with PBS. Membranes were incubated with ECL Plus western Blotting Detection System (Amersham Bioscience) and developed using BioMax MR Films (Kodak).

Protein expression and purification

Yeast Rpb1-9Myc (RNAP-II from yeast) was purified from 10 mg of whole cell extract using magnetic beads pre-bound with anti-MYC antibodies (μMACS c-myc; Miltenyi Biotec) following the recommendations of the kit. hRPB1 (RNAP-II from human cells) was purified using 10mg of whole cell extract from HEK293T cultures with an antibody against total RNAP-II (H224, Santa Cruz, Sc-9001). Yeast Ssu72, Pph21, Msg5, and Cdc14 and hCDC14A as well as its phosphatase-inactive form were expressed and purified from *e.coli* cultures carrying the expression plasmids. A litre of bacterial cells were grown overnight (50ml of lysogeny broth (LB) plus ampicilin) at 37°C, to reach $OD_{600\ nm}$ of 1.0. Cells were then induced with 0.5 M IPTG for 6h. Following centrifugation, the pellet was resuspended in 30 ml lysis buffer (0.1% Triton X-100, PBS and 1mgr/ml lysozyme) and incubated at room temperature for 15min. Cells were sonicated three times for 30s on ice, the extract was centrifuged at 14.000 rpm for 30 min and the supernatant was collected. The supernatant was further incubated with 1ml pre-equilibrated GST beads for 30min at room temperature, centrifuged and washed three times with 5ml lysis buffer, then twice with 5ml lysis buffer plus 0.5M NaCl and Triton X-100, followed by a final set of three washes with 5ml lysis buffer. Elution was done by incubation with 5ml of glutathione for 5min.

Phosphatase *in vitro* assay

Yeast Rpb1 was incubated with Ssu72/ Pph21/Msg5/Cdc14/Cdc14CS for 45 minutes at 30°C in phosphatase buffer (25mM HEPES, 150mM NaCl, 0.1mgr/ml BSA and 2mM $MnCl_2$). Human Rpb1 was incubated with CDC14A/CDC14A-CS for 45 minutes at 30°C in phosphatase buffer (25mM HEPES, 150mM NaCl, 0.1mgr/ml BSA and 2mM $MnCl_2$).

Chromatin Immunoprecipitation (ChIP)

Standard ChIP was performed with modifications as follows. Yeast cultures (50ml) were grown to OD_{600nm} 0.5. Cells were crosslinked with 1% folmaldehyde for 15 minutes at RT, then glycine was added to 125mM, cells were centrifuged, washed with PBS, and stored at −80°C. Cells were resuspended in 100 μl IP-lysis buffer containing protease inhibitors and 1mM PMSF, then subjected to bead-beating. After recovery of spheroplasts, the ChIP protocol was continued as described (*2*). SYBr green real-time PCR was used for quantification. Primer sequences are available on request. ChIP values were calculated as a percentage of material immunoprecipitated (ChIP/input). The data is present as a fold enrichment setting the % immunoprecipitated in the control to 1 (*CEN4* or *ACT1*). All statistical calculations were performed using the GraphPad PRISM Version 5 statistical software package. A list of primers used for this study can be found in Table S2.

Microscopy and determination of segregation defects

Yeast cells with GFP-tagged proteins were analyzed by fluorescence microscopy after DAPI or propidium iodide staining. Images were collected on a Leica IRB using a Hamamatsu C4742-95 digital camera and OpenLab software (Improvision). Imaging was done in

antifade medium (Molecular Probes, Eugene, OR) at room temperature. In each chromosome tag segregation/separation experiment an average of 200 cells were counted for each time point and condition.

RNA methods

For total RNA isolation, the cell extracts were cleaned from proteins and DNA using TRIZOL® Reagent (Invitrogene). Then RNeasy Kit (Quiagen), was used following the manufacturer's instructions to ensure the total degradation of DNA in the sample. ThermoScript™ RT-PCR System for First-Strand cDNA Synthesis (Invitrogene) was used. Quantifications were done using SYBr green real-time PCR. Primer sequences are available on request. All statistical calculations (including p-value calculations) were performed using the GraphPad PRISM Version 5 statistical software package.

Microarray transcription analysis

The arrays used were from Affymetrix (Santa Clara, CA) (PN 520055). Total RNA was isolated by hot phenol extraction. Three replicate hybridizations (biological) of total RNA were performed. Probe sequences were aligned to the genome sequence of *S. cerevisiae* strain S288c. The Affymetrix, Tiling Analysis Software (TAS) application was used for exploratory data analysis (MVA plots & Histograms) and to generate intensities and p-values. Yeast tiling array cel files were uploaded and normalised with quantile normalisation and intensity scaling. Intensity analysis was used to output the relative signal intensities on a −10 log 10 scale and the corresponding p-values were calculated with a 2-sided test. The p-values were adjusted for multiple testing with the Benjamini Hochberg method in the R package p.adjust. Perl scripts were written to convert the signals and adjusted p-values into bigwig format for visualisation on the UCSC. Parteck Genomic suite was used for exploratory data analysis (PCA plots) and to output the raw signal values for the control and treatment groups. The cel files were uploaded and normalised with Quantile normalisation and RMA background correction.The tiling array data has been deposited on the Gene Expression Omnibus and can be accessed using the number GSE31020 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE31020).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Orphanides G, Reinberg D. A unified theory of gene expression. Cell. 2002; 108:439–451. [PubMed: 11909516]
2. Visintin R, et al. The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation. Mol Cell. 1998; 2:709–718. [PubMed: 9885559]
3. Stegmeier F, Amon A. Closing mitosis: the functions of the Cdc14 phosphatase and its regulation. Annu Rev Genet. 2004; 38:203–232. [PubMed: 15568976]

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4. D'Amours D, Stegmeier F, Amon A. Cdc14 and condensin control the dissolution of cohesin-independent chromosome linkages at repeated DNA. Cell. 2004; 117:455–469. [PubMed: 15137939]
5. Shou W, et al. Exit from mitosis is triggered by Tem1-dependent release of the protein phosphatase Cdc14 from nucleolar RENT complex. Cell. 1999; 97:233–244. [PubMed: 10219244]
6. Visintin R, Hwang ES, Amon A. Cfi1 prevents premature exit from mitosis by anchoring Cdc14 phosphatase in the nucleolus. Nature. 1999; 398:818–823. [PubMed: 10235265]
7. Stegmeier F, Visintin R, Amon A. Separase, polo kinase, the kinetochore protein Slk19, and Spo12 function in a network that controls Cdc14 localization during early anaphase. Cell. 2002; 108:207–220. [PubMed: 11832211]
8. Torres-Rosell J, Machin F, Jarmuz A, Aragon L. Nucleolar segregation lags behind the rest of the genome and requires Cdc14p activation by the FEAR network. Cell Cycle. 2004; 3:496–502. [PubMed: 15004526]
9. Sullivan M, Higuchi T, Katis VL, Uhlmann F. Cdc14 phosphatase induces rDNA condensation and resolves cohesin-independent cohesion during budding yeast anaphase. Cell. 2004; 117:471–482. [PubMed: 15137940]
10. Machin F, et al. Transcription of ribosomal genes can cause nondisjunction. J Cell Biol. 2006; 173:893–903. [PubMed: 16769819]
11. Tomson BN, D'Amours D, Adamson BS, Aragon L, Amon A. Ribosomal DNA transcription-dependent processes interfere with chromosome segregation. Mol Cell Biol. 2006; 26:6239–6247. [PubMed: 16880532]
12. Clemente-Blanco A, et al. Cdc14 inhibits transcription by RNA polymerase I during anaphase. Nature. 2009; 458:219–222. [PubMed: 19158678]
13. Smith JS, Boeke JD. An unusual form of transcriptional silencing in yeast ribosomal DNA. Genes Dev. 1997; 11:241–254. [PubMed: 9009206]
14. Bryk M, et al. Transcriptional silencing of Ty1 elements in the RDN1 locus of yeast. Genes Dev. 1997; 11:255–269. [PubMed: 9009207]
15. Straight AF, et al. Net1, a Sir2-associated nucleolar protein required for rDNA silencing and nucleolar integrity. Cell. 1999; 97:245–256. [PubMed: 10219245]
16. Imai S, et al. Sir2: an NAD-dependent histone deacetylase that connects chromatin silencing, metabolism, and aging. Cold Spring Harb Symp Quant Biol. 2000; 65:297–302. [PubMed: 12760043]
17. Houseley J, Kotovic K, El Hage A, Tollervey D. Trf4 targets ncRNAs from telomeric and rDNA spacer regions and functions in rDNA copy number control. Embo J. 2007; 26:4996–5006. [PubMed: 18007593]
18. Egloff S, Murphy S. Cracking the RNA polymerase II CTD code. Trends Genet. 2008; 24:280–288. [PubMed: 18457900]
19. Mayan M, Aragon L. Cis-interactions between non-coding ribosomal spacers dependent on RNAP-II separate RNAP-I and RNAP-III transcription domains. Cell Cycle. 9:4328–4337. [PubMed: 20980822]
20. Jaspersen SL, Morgan DO. Cdc14 activates cdc15 to promote mitotic exit in budding yeast. Curr Biol. 2000; 10:615–618. [PubMed: 10837230]
21. Jiang Y. Regulation of the Cell Cycle by Protein Phosphatase 2A in Saccharomyces cerevisiae. Microbiol Mol Biol Rev. 2006; 70:440–449. [PubMed: 16760309]
22. Doi K, et al. MSG5, a novel protein phosphatase promotes adaptation to pheromone response in S. cerevisiae. EMBO J. 1994; 13:61–70. [PubMed: 8306972]
23. Krishnamurthy S, He X, Reyes-Reyes M, Moore C, Hampsey M. Ssu72 Is an RNA polymerase II CTD phosphatase. Mol Cell. 2004; 14:387–394. [PubMed: 15125841]
24. David L, et al. A high-resolution map of transcription in the yeast genome. Proc Natl Acad Sci U S A. 2006; 103:5320–5325. [PubMed: 16569694]
25. Wang BD, Butylin P, Strunnikov A. Condensin function in mitotic nucleolar segregation is regulated by rDNA transcription. Cell Cycle. 2006; 5:2260–2267. [PubMed: 16969110]

26. Freeman L, Aragon-Alcaide L, Strunnikov A. The condensin complex governs chromosome condensation and mitotic transmission of rDNA. J Cell Biol. 2000; 149:811–824. [PubMed: 10811823]
27. Cuylen S, Metz J, Haering CH. Condensin structures chromosomal DNA through topological links. Nat Struct Mol Biol. 2011; 18:894–901. doi:nsmb.2087 [pii]10.1038/nsmb.2087. [PubMed: 21765419]
28. Lengronne A, et al. Cohesin relocation from sites of chromosomal loading to places of convergent transcription. Nature. 2004; 430:573–578. doi:10.1038/nature02742 nature02742 [pii]. [PubMed: 15229615]
29. Chan JN, et al. Perinuclear cohibin complexes maintain replicative life span via roles at distinct silent chromatin domains. Dev Cell. 2011; 20:867–879. doi:S1534-5807(11)00206-1 [pii]10.1016/j.devcel.2011.05.014. [PubMed: 21664583]
30. Dulev S, et al. Essential global role of CDC14 in DNA synthesis revealed by chromosome underreplication unrecognized by checkpoints in cdc14 mutants. Proc Natl Acad Sci U S A. 2009; 106:14466–14471. doi:0900190106 [pii]10.1073/pnas.0900190106. [PubMed: 19666479]
31. D'Ambrosio C, Kelly G, Shirahige K, Uhlmann F. Condensin-dependent rDNA decatenation introduces a temporal pattern to chromosome segregation. Curr Biol. 2008; 18:1084–1089. [PubMed: 18635352]
32. Baxter J, et al. Positive supercoiling of mitotic DNA drives decatenation by topoisomerase II in eukaryotes. Science. 2011; 331:1328–1332. doi:331/6022/1328 [pii]10.1126/science.1201538. [PubMed: 21393545]
33. Chafin DR, Guo H, Price DH. Action of alpha-amanitin during pyrophosphorolysis and elongation by RNA polymerase II. J Biol Chem. 1995; 270:19114–19119. [PubMed: 7642577]
34. Bembenek J, H Y. Regulation of the anaphase-promoting complex by the dual specificity phosphatase human Cdc14a. J. Biol. Chem. 276:48237–42. [PubMed: 11598127] J. Biol. Chem. 2001; 276:48237–48242. [PubMed: 11598127]
35. Mailand N, et al. Deregulated human Cdc14A phosphatase disrupts centrosome separation and chromosome segregation. Nat Cell Biol. 2002; 4:317–322. [PubMed: 11901424]
36. Paulsen MT, et al. The p53-targeting human phosphatase hCdc14A interacts with the Cdk1/cyclin B complex and is differentially expressed in human cancers. Mol Cancer. 2006; 5:25. [PubMed: 16784539]
37. Kaiser BK, Zimmerman ZA, Charbonneau H, Jackson PK. Disruption of centrosome structure, chromosome segregation, and cytokinesis by misexpression of human Cdc14A phosphatase. Mol Biol Cell. 2002; 13:2289–2300. [PubMed: 12134069]
38. Bassermann F, et al. The Cdc14B-Cdh1-Plk1 axis controls the G2 DNA-damage-response checkpoint. Cell. 2008; 134:256–267. [PubMed: 18662541]

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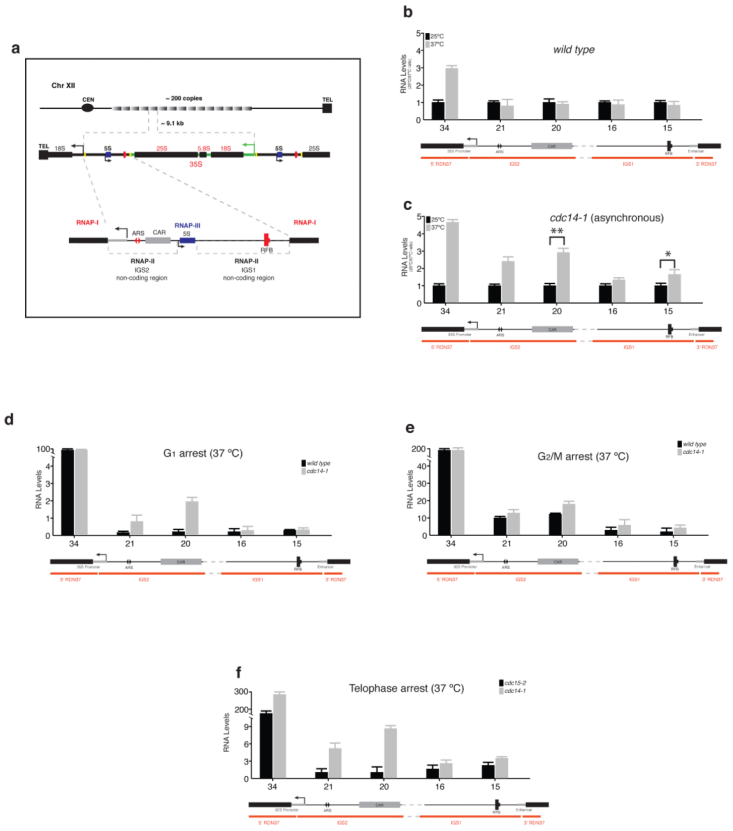

Figure 1.

Cdc14 is required for rDNA silencing.

(a) Schematic representation of the intergenic spacer regions (IGS1/IGS2). Specific features of these regions include a replication origin (ARS), a cohesin binding site (CAR) and a replication fork barrier sequence (RFB). (b) RT-PCR-based analysis of transcripts within IGS regions in wild type cells grown at 25°C and 37°C. Transcription levels at 37°C relative to 25°C are shown for each position (mean ± S.E.M. n=3). (c) RT-PCR-based analysis of transcripts within IGS regions in *cdc14-1* cells grown at 25°C and 37°C. Transcription levels at 37°C relative to 25°C are shown for each position (mean ± S.E.M. n=7). P-values; *0.016, **0.005; student's t-test. (d) RT-PCR-based analysis of transcripts within IGS regions in wild type and *cdc14-1* cells arrested in G1 at 25°C (with α-factor) and shifted to 37°C for two additional hours. (mean ± S.E.M. n=3). (e) RT-PCR-based analysis of transcripts within IGS regions in wild type and *cdc14-1* cells arrested in metaphase at 25°C (with nocodazole) and shifted to 37°C (mean ± S.E.M. n=3). (f) RT-PCR-based analysis of transcripts within IGS regions in *cdc15-2* and *cdc14-1* cells grown at 25°C and shifted to 37°C for three hours to reach a telophase arrest. (mean ± S.E.M. n=3).

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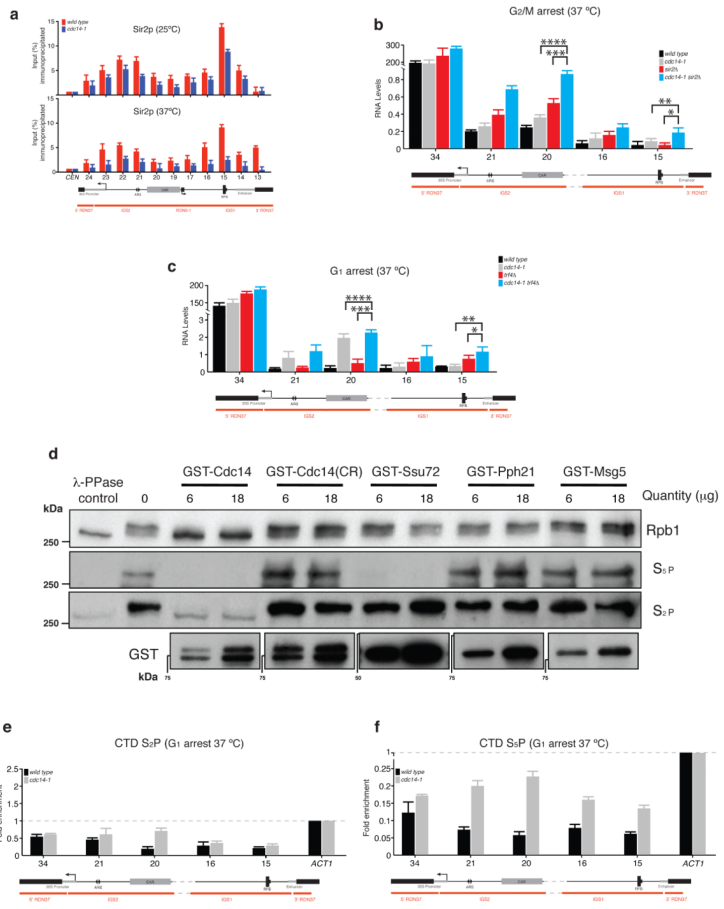

Figure 2.

Cdc14 is an RNAP-II CTD phosphatase.

(a) Chromatin immuno-precipitation (ChIP) analysis of Sir2p binding at 25°C (top graph) and 37°C (bottom graph) in *cdc14-1* and wildtype cells within the rDNA IGS regions of chromosome XII. Fold enrichment relative to *CEN4* is shown for each location (mean ± S.E.M. n=4). (b) RT-PCR-based analysis of transcripts within IGS regions in wild type, *sir2Δ*, *cdc14-1* and *sir2Δ cdc14-1* cells arrested in metaphase at 25°C (with nocodazole) and shifted to 37°C for two additional hours. (mean ± S.E.M. n=8). P-values; *0.036, **0.047, ***0.016, ****0.021; student's t-test. (c) RT-PCR-based analysis of transcripts within IGS regions in wild type, *trf4Δ*, *cdc14-1* and *trf4Δ*/*cdc14-1* cells arrested in G1 at 25°C (with α-factor) and shifted to 37°C two additional hours. (mean ± S.E.M. n=8). P-values; *0.031, **0.028, ***0.020, ****0.051; student's t-test. (d) Purified Rpb1 was incubated with buffer, purified GST–Cdc14, a catalytically inactive mutant GST–Cdc14C/R (C283S, R289A), GST-Ssu72, GST-Pph21 or GST-Msg5, then analysed by immuno-blotting using antibodies against CTD S_5 and S_2 phosphorylation. Quantity (μg) of purified protein added to each reaction is shown. (e) Chromatin immuno-precipitation (ChIP) analysis of RNAP-II phosphorylated at CTD serine-2 (S_2P) within IGS regions in wild type and *cdc14-1* cells arrested in G1 at 25°C (with α-factor) and shifted to 37°C for two additional hours. Fold enrichment relative to *ACT1* locus is shown (mean ± S.E.M. n=3). (f) ChIP analysis of RNAP-II phosphorylated at CTD serine-5 (S_5P) within IGS regions in wild type and *cdc14-1* cells arrested in G1 at 25°C (with α-factor) and shifted to 37°C for two additional hours. Fold enrichment relative to *ACT1* locus is shown (mean ± S.E.M. n=3). Uncropped images of blots are shown in Supplementary Fig. S4.

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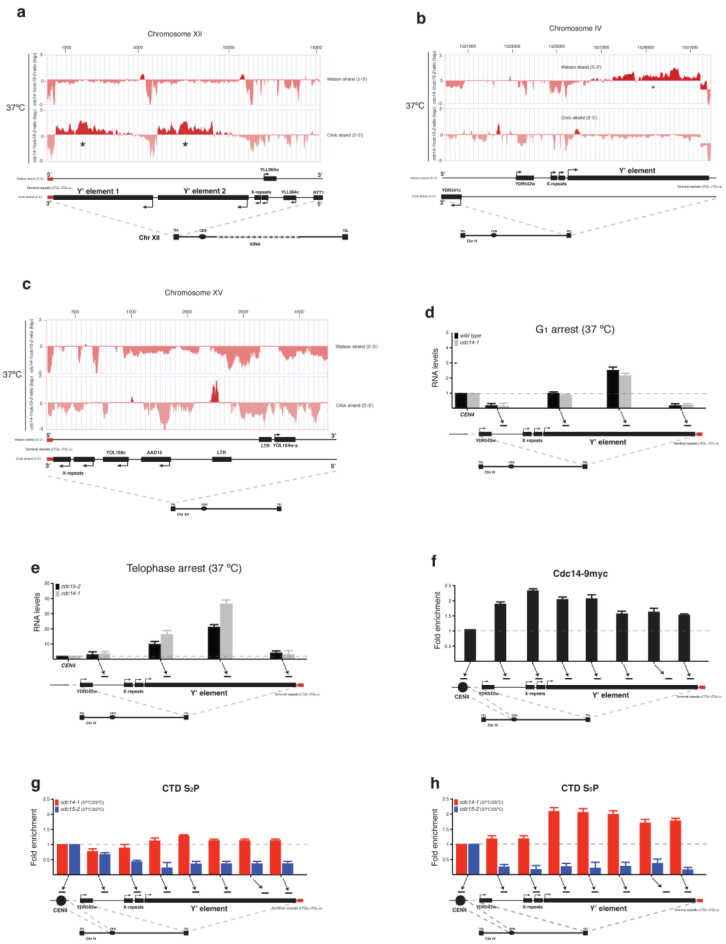

Figure 3.

Cdc14 represses transcription of sub-telomeric Y'elements.

(a) Yeast tiling array intensities of the distal 15kb of the left telomere of chromosome XII containing two Y' elements (*x* axis). Plotted is the $\log_2$ ratio of transcript abundance in *cdc14-1* relative to *cdc15-2* (red) at 37°C for the Watson and Crick strands (*y* axis). Y' elements are indicated (*). Transcript abundance for this region at 25°C can be found in Suppl. Fig. 3. (b) Yeast tiling array intensities as in (a) for the distal 10kb of the right telomere region of chromosome IV. Y' elements are indicated (*). (c) Yeast tiling array intensities as in (a) for the distal 5kb of the left telomere region of chromosome IV. The region does not contain Y'elements. Transcript abundance for this region at 25°C can be found in Suppl. Fig. 2. (d) RT-PCR-based analysis of transcripts within the region of the right telomere of chromosome IV in wild type and *cdc14-1* cells arrested in G1 at 25°C (with α-factor) and shifted to 37°C or maintained at 25°C for two additional hours. RNA levels relative to *CEN4* are shown for each position (mean ± S.E.M. n=3). (e) RT-PCR-based analysis of transcripts within the region of the right telomere of chromosome IV in *cdc15-2* and *cdc14-1* cells grown at 25°C and shifted to 37°C for three hours to reach a telophase arrest. RNA levels relative to *CEN4* are shown for each position (mean ± S.E.M. n=3). (f) Chromatin immuno-precipitation (ChIP) analysis of Cdc14p binding within the sub-telomeric region of the right telomere of chromosome IV. Fold enrichment relative to *CEN4* is shown for each position (mean ± S.E.M. n=3). (g) ChIP analysis of CTD serine-2 phosphorylation (S_2P) at 37°C relative to 25°C in *cdc14-1* and *cdc15- 2* cells within the sub-telomeric region of the right telomere of chromosome IV. Fold enrichment relative to *CEN4*

is shown for each position (mean ± S.E.M. n=3). (h) ChIP analysis of CTD serine-5 phosphorylation (S_5P) as in (g). Fold enrichment relative to *CEN4* is shown for each position (mean ± S.E.M. n=3).

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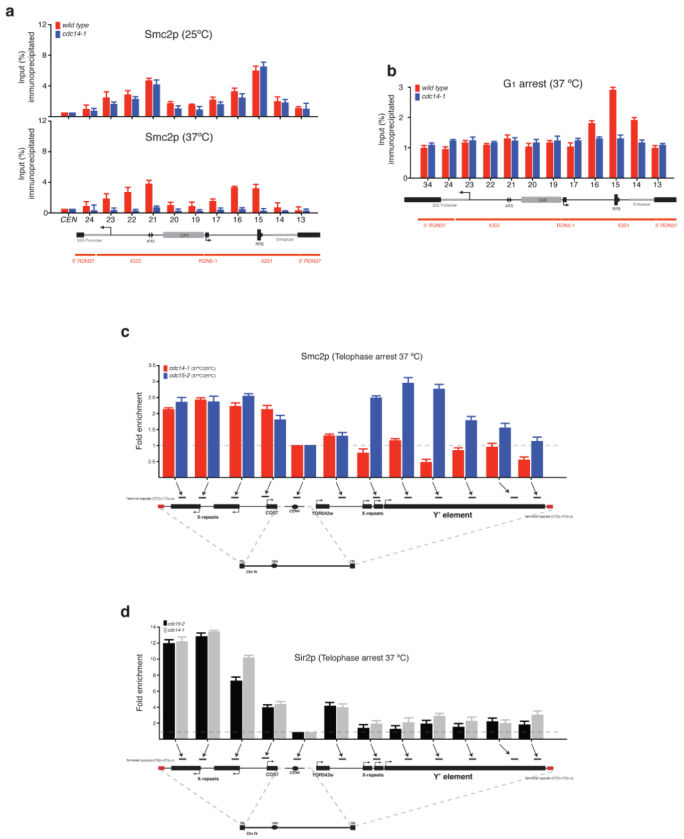

Figure 4.

Cdc14 recruits Condensin to telomeres.

(a) Chromatin immuno-precipitation (ChIP) analysis of Smc2p binding at 25°C (top graph) and 37°C (bottom graph) in *cdc14-1* and wildtype cells within the rDNA IGS regions of chromosome XII. Fold enrichment relative to *CEN4* is shown for each location. (mean ± S.E.M. n=3). (b) ChIP analysis of Condensin subunit Smc2 binding within IGS regions in wild type and *cdc14-1* cells arrested in G1 at 25°C (with α-factor) and shifted to 37°C for two additional hours. Data is shown as a percentage of input immunoprecipitated for each position (mean ± S.E.M. n=4). (c) ChIP analysis of Condensin subunit Smc2 binding at 37°C relative to 25°C in *cdc14-1* and *cdc15-2* cells within the sub-telomeric (left, *TEL4L*, and right, *TEL4R*, arms) and centromeric (*CEN4*) regions of chromosome IV. Fold enrichment relative to *CEN4* is shown for each position (mean ± S.E.M. n=3). (d) ChIP analysis of Sir2p binding at 37°C relative to 25°C in *cdc14-1* and *cdc15-2* cells within the *TEL4L*, *CEN4* and *TEL4R* regions of chromosome IV. Fold enrichment relative to *CEN4* is shown for each location (mean ± S.E.M. n=3).

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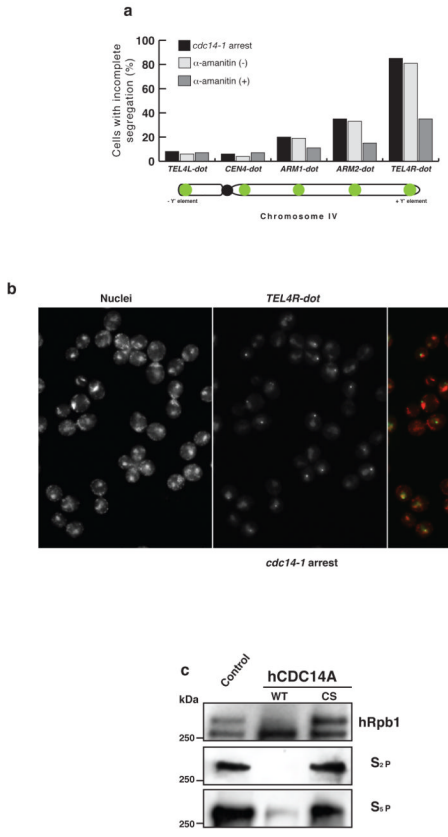

Figure 5.

Segregation of telomeres with Y'-element requires Cdc14.

(a) Segregation analysis of *cdc14-1* mutants strains carrying a tetO-dot close to *CEN4* (*CEN4*-dot), left telomere (*TEL4L*–dot), right telomere (*TEL4R*–dot) or the middle of chromosome IV (*ARM1*-dot and *ARM2*-dot) (as indicated). Cells were released from G1 (α-factor) into telophase arrest at 37°C (*cdc14-1* arrest), before treatment with the RNAP-II inhibitor α-amanitin (66μg/ml). Segregation of tetO-dots in the arrest (*cdc14-1* arrest) and after α-amanitin (RNA Polymerase II −) or mock treatment (RNA Polymerase II +) was scored and quantified (Three hundred cells were scored). (b) Comparative micrographs showing lack of segregation of *TEL4R*-dots in the *cdc14-1* telophase arrest. Scale bar 10μm. (c) Purified human Rpb1 was incubated with hCDC14A or hCDC14ACS (phosphatase-dead) proteins purified from bacterial cells. Western blot analysis of CTD S_2P and S_5P after incubation is shown. Uncropped images of blots are shown in Supplementary Fig. S4.