HHS Public Access

Author manuscript

Nat Struct Mol Biol. Author manuscript; available in PMC 2012 June 01.

Published in final edited form as:

Nat Struct Mol Biol. ; 18(12): 1351–1357. doi:10.1038/nsmb.2151.

The Chp1-Tas3 core is a multifunctional platform critical for gene silencing by RITS

Thomas Schalch[1], **Godwin Job**[2], **Sreenath Shanker**[2], **Janet F. Partridge**[2], and **Leemor Joshua-Tor**[1,3]

[1]Keck Structural Biology Laboratory, Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724

[2]Department of Biochemistry, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, USA

[3]Howard Hughes Medical Institute

Abstract

RNA interference (RNAi) is critical for the assembly of heterochromatin at fission yeast centromeres. Central to this process is the RNA-induced Initiation of Transcriptional gene Silencing (RITS) complex, which physically anchors small non-coding RNAs to chromatin. RITS includes Ago1, the chromodomain protein Chp1, and Tas3, which bridges between Chp1 and Ago1. Chp1 is a large protein with, apart from its chromodomain, no recognizable domains. Here we describe how the structured C-terminal half of Chp1 binds the Tas3 N-terminal domain, revealing Chp1's tight embrace of Tas3. The structure also reveals a PIN domain at the C-terminal tip of Chp1 that controls subtelomeric transcripts through a post-transcriptional mechanism. We suggest that the Chp1-Tas3 complex provides a solid and versatile platform to recruit both RNAi-dependent and RNAi-independent gene-silencing pathways for locus-specific regulation of heterochromatin.

Introduction

Tightly controlled establishment and maintenance of heterochromatin is crucial for proper chromosome segregation, transcriptional control and many other chromosome associated processes. In the fission yeast *Schizosaccharomyces pombe*, centromeres, telomeres, and the mating type locus are heterochromatic. These regions are governed by a complex system that relies on RNA-guided processes as well as DNA-specific binding events and

Correspondence: Leemor Joshua-Tor, leemor@cshl.edu, 516-367-8821 (tel), 516-367-8873 (fax); Janet F. Partridge, janet.partridge@stjude.org, 901-595-2679 (tel), 901-525-8025.

Accession codes: The atomic coordinates and structure factors for the Chp1-Tas3 complex have been deposited in the Protein Data Bank with accession number 3TIX.

Author contributions: T.S. designed, conducted and interpreted the *in vitro*, structural studies and bioinformatics analyses and wrote the paper. G.J. generated yeast strains and performed transcript analyses and westerns. S.S. generated yeast strains, performed transcript analyses, westerns and ChIP experiments. J.F.P generated yeast strains, designed and interpreted experiments and wrote the paper. L.J. designed and interpreted experiments and wrote the paper.

modification of chromatin[1,2]. Heterochromatin formation and gene silencing at the centromere is highly dependent on transcription of the pericentromeric dh and dg repeats by RNA polymerase II (Pol II) and the subsequent recruitment of the RNAi machinery. The RNAi system associated with heterochromatin consists of the ribonuclease III (RNase III)–like enzyme Dicer (Dcr1) generating small RNA molecules of 20-30 nucleotides (nt) in length from centromeric transcripts that are amplified by an RNA-dependent RNA polymerase Rdp1[3]. These small RNA products are loaded into the Argonaute family protein Ago1. Processing of transcripts at the centromere by the RNAi machinery targets them for degradation through post-transcriptional gene silencing mechanisms that operate in cis (cis-PTGS)[4-6]. RNAi is also cruicial for the recruitment of chromatin factors by contributing to the nucleation of the histone 3 lysine-9 (H3K9) specific Clr4 histone methyltransferase and its associated complex (ClrC)[7-9].By establishing H3K9 methylation, Clr4 provides the platform for the assembly of a repressive chromatin structure consisting of the HP1 homologous chromodomain proteins Chp2 and Swi6, histone deacetylases, chromatin remodeler and histone chaperone complexes[10]. At the centromere there are thus two self-enforcing systems acting in parallel, on one hand through transcriptional gene silencing (TGS) and on the other through cis-PTGS.

At the nexus of these two systems the RNA-induced initiation of Transcriptional Gene Silencing (RITS) complex has been identified as a central player, linking the RNAi pathway with heterochromatin formation[11,12]. RITS contains the chromodomain protein Chp1, the GW protein Tas3 and the argonaute protein Ago1 in complex with a small RNA. Chp1 has been shown to bind the heterochromatic histone H3K9 methyl mark with high affinity[13], while Ago1 targets RNA transcripts via its bound siRNA[14,15]. The cooperation of these two activities mediated through assembly of the RITS complex is essential for the establishment and maintenance of heterochromatin.Tas3 provides the backbone of the RITS complex, binding to Chp1 through its N-terminal domain (NTD), to Ago1 by means of its GW domain, and features a multimerization domain at its C terminus[16-20]. RITS function is critical for heterochromatin formation because of its crucial role in recruitment of the RNA-dependent RNA Polymerase complex (RDRC) for amplification of siRNA precursor transcripts, as well as in the recruitment of the Clr4 methyltransferase Complex (ClrC) for the deposition of methyl marks[7-9,21]. So far, the molecular details of these recruitment processes remain unclear.Chp1 is a protein of 960 amino acid residues with the chromodomain located at its N terminus[22,23]. Its C terminus has been shown to be required for the interaction with Tas3[16]. In order to better understand the structural and functional role of the RITS complex, we have identified the folded core of the Chp1-Tas3 sub-complex and determined its structure through X-ray crystallography. The structure reveals Chp1's tight embrace of Tas3, and the unexpected presence of a C-terminal PIN domain in Chp1. We show that the PIN domain contributes significantly to silencing of subtelomeric transcripts through post-transcriptional gene silencing in a mechanism independent of RNAi.

Results

The Chp1-Tas3 heterodimer has a structured core

The complex of full length Chp1 with Tas3 (1-309), deleting the aggregation prone Tas3 C-terminal α-helical motif was obtained by co-expression of the two subunits in the Multibac baculovirus expression system[24]. In this heterologous expression system, Chp1 and Tas3 behave as an obligatory heterodimer, since Tas3 stability is entirely dependent on co-expression with Chp1. Chp1 can be obtained individually as a OneStrep-SumoStar (OSS) fusion protein, but aggregates upon removal of the tag. Chp1 and Tas3 are both predicted to contain highly flexible regions (Supplementary Figure 1a). We therefore used limited proteolysis on the purified complex in order to identify stably folded domains. These experiments revealed that the Tas3 N-terminal domain (NTD) and the C-terminal half of Chp1 (residues 504-960) form the stable core of the complex (Figure 1a and Supplementary Figure 1b). The boundaries determined for Chp1 agree well with the region identified to interact with Tas3[16]. The C-terminal boundary of the Tas3 fragment could not be determined accurately due to heterogeneity in the proteolytic fragments, therefore the fragment (residues 9-83) previously shown to be sufficient in maintaining Chp1-Tas3 interaction was used[19]. The Chp1(504-960)-Tas3(9-83) core was expressed with an OSS tag on the N terminus of either Tas3 or Chp1. A mass of 93 kDa was measured by multiangle light scattering (MALS) for the OSS-Chp1 tagged complex, corresponding closely to the 87.7 kDa expected for a 1:1 stoichiometry. The OSS-Tas3 tagged construct (Supplementary Figure 2) yielded well diffracting crystals that were used to solve the structure by the Single Anomalous Dispersion method (see Supplementary Methods). In the crystal structure we observe two molecules in the asymmetric unit with a critical crystal contact mediated by the OSS tag (Supplementary Figure 3).

Structure of the Chp1-Tas3 core

The structure of Chp1 and Tas3 reveals a tight, elongated complex, resembling the hull of a ship (Figure 1b), with dimensions 116 Å × 44 Å × 36 Å. The complex can be divided into four subdomains: three distinctive mixed α/β domains in Chp1, and an α-helical bundle in Tas3, which is completely engulfed by the first two domains of Chp1 and the linker between them. The two molecules in the asymmetric unit are very similar (overall RMSD of 1.25 Å), except for a difference along their long axis that is manifested in a bend of 7 degrees distributed across the linker domain residues 666-672 of Chp1.Structural similarity searches for individual domains identified related structures for each (Supplementary Figure 4). However, the overall arrangement and protein-protein interface observed here represents a novel architecture. Domain I of Chp1 (aa 515-651) is a β-barrel domain, and shows strong structural similarity to the SPOC domain of the transcriptional corepressor SHARP [25]. The Chp1 SPOC domain binds the first α-helix (α1) of Tas3 (residues 9-17),embedding it in a deep hydrophobic groove formed by the Chp1 SPOC domain and the first α-helix of the Chp1 linker domain (aa 654-661) (Supplementary Figure 5). The Tas3 residues 18-23 following the α1 helix bind to the Chp1 SPOC domain in an extended conformation, forming an interface reminiscent of the one used by the SPOC domain of SHARP for interaction with SMRT/NCoR.

The Chp1 SPOC domain is connected to the second Chp1 domain (aa 684-808) by a 33 amino-acid linker that forms two Tas3 flanking helices and bridges across the Tas3 N-terminal domain (NTD). The α2 and α3 helices of Tas3 fold as a helical hairpin that is "crosslinked" at its base by α1 and surrounded by four shorter α-helices of Chp1, comprised by the α6 and α7-helices of the linker domain, the α3-helix of the SPOC domain and α8 of Domain II (Supplementary Figure 6). It is interesting to note that GW182 proteins, which share homology with Tas3 in their GW motifs, are predicted to have a coiled-coil domain at the N terminus, although it has not been linked to function. It is also notable that the silencing protein Sir4 in *S. cerevisiae* forms a coiled coil that interacts with the C-terminal half of Sir3[26]. Domain II of Chp1 is a member of the Rossmann fold family, resembling eukaryotic phosphatases and receiver domains of bacterial phosphotransferase signaling systems. In the complex observed here, one side of this domain, which includes the putative active site region interacts extensively with Tas3 α2 and α3.

The key function of the Tas3-NTD in complex formation observed here is in excellent agreement with previous work showing that deletion of Tas3 residues 10-24 including helix α1, abolishes Chp1-Tas3 complex formation, leading to extensive loss of heterochromatic silencing[19]. In addition, two-hybrid mapping revealed that residues encompassing the SPOC domain of Chp1 (aa 505-661) suffice for interaction with Tas3 (Supplementary Figure 7). The interaction between Domain II and Tas3 contributes 42% of the buried surface area of the complex. However, we could not verify the interaction between Domain II and Tas3 in yeast two-hybrid assays due to instability of the Domain II fusion protein (Supplementary Figure 7).

The tight embrace of Tas3 by the SPOC domain, the linker, and Domain II make up the interaction surface between Chp1 and Tas3, resulting in a substantial buried surface area of 4890 $Å^2$. PISA analysis yields a ΔG_{solv} of -39 $kcalmol^{-1}$, indicative of a strong hydrophobic interaction[27], comprising of 59 residues of Tas3 and 65 residues of Chp1 with 17 hydrogen bonds and 4 salt bridges (Supplementary Figure 5,6). Buried within the relatively flat interface between Domain II and Tas3 α3 we observe a cavity containing two strong spherical peaks of electron density coordinated by Arg69 of Tas3.These were interpreted as chloride ions based on the chemical environment, the fit to the electron density, and buffer ingredients. There is a possibility that the chloride ions substitute for parts of a functionally important small molecule ligand that might occupy this cavity under different conditions. This hypothesis was tested by introducing Tas3 mutations R69A and C68A into *S. pombe*. However no discernible effect on transcript levels at centromeres could be observed in these mutants (Supplementary Figure 8).

The extensive interface formed between Tas3 and the SPOC domain, linker and Domain II of Chp1 suggests that the complex is an obligate heterodimer. This is supported by the observation that Tas3's stability in the absence of Chp1 is highly dependent on a C-terminal TAP tag[16,19]. Since no protein stability issues have been observed for Tas3 in the absence of Ago1, neither *in vivo* nor i*n vitro*, the tight interface between Chp1 and Tas3 lends support to the model proposing that Chp1 and Tas3 form a stable and possibly polymeric[20] platform, with Ago1 being a more loosely associated component of the complex[16].

Chp1 contains a PIN domain at its C terminus

Domain III of Chp1 (aa 809-960) is independent of the Chp1-Tas3 interface, and shares a strong structural similarity with PIN domains (Figure 2a and Supplementary Figure 4). PIN domains are abundant throughout all organisms. They function as nucleases or as protein-protein interaction modules[28-30]. Specifically, the Chp1 PIN domain is most similar to the PIN domains of SMG5/EST1B and SMG6/EST1A[31], proteins involved in the nonsense mediated mRNA decay (NMD) pathway[32-34] and, interestingly, in telomere maintenance in metazoan organisms[35,36]. The PIN domain of human SMG5 has also been implicated in recruitment of the phosphatase PP2A to UPF1[37,38]. Furthermore, it shares similarity with the PIN domain of the exosome subunit Dis3/Rrp44, which apart from its endonuclease function is also critical for association with the exosome core[30,39].

The PIN domain silences *tlh* transcripts post-transcriptionally

Chp1 is important for both the establishment and maintenance of heterochromatin in fission yeast[16,22,23,40,41].In wild type fission yeast, the assembly of centromeric repeats and of subtelomeric genes into silenced domains occurs via both transcriptional and post-transcriptional gene silencing[5,6,42]. Cells lacking RITS components accumulate transcripts from pericentromeric repeats[3,11,23]. At telomeres, silencing of the subtelomeric telomere-linked helicase (*tlh*)genes is derepressed following deletion of Chp1 or Tas3, but not of other RNAi components, suggesting that Chp1 and Tas3 have additional roles in silencing *tlh* transcripts outside the context of RITS[19,40]. In order to investigate the function of the PIN domain of Chp1, DNA sequences encoding residues 809-960 encompassing the PIN domain were deleted from the genomic *chp1* allele in fission yeast to generate *chp1*Δ*C*. Real time PCR analyses of transcripts derived from the *dg* or *dh* outer repeats of the centromere (Figure 3a) demonstrated that whereas cells lacking Chp1 displayed strong accumulation of transcripts, centromeric heterochromatin was unaffected by the loss of the PIN domain in *chp1*Δ*C* strains (Figure 3b,c,d Supplementary Figure 9). In marked contrast, subtelomeric *tlh* transcripts (Figure 3a) accumulated in *chp1*Δ*C* strains (Figure 3b,e Supplementary Figure 9). These data suggest that the PIN domain of Chp1 contributes to the RNAi independent function observed for Chp1 in the regulation of subtelomeric chromatin. Next we asked whether the PIN domain was involved in chromatin-mediated transcriptional gene silencing or post-transcriptional regulation of subtelomeric sequences. If the PIN domain affects transcriptional regulation, RNA polymerase II (Pol II) occupancy might be expected to increase on subtelomeric sequences *chp1*Δ*C* strains. ChIP with anti-Pol II antibodies revealed that Pol II occupancy on *tlh* sequences was relatively unaffected by loss of $chp1^{+}$. In contrast, at centromeres, Pol II occupancy increased in strains lacking $chp1^{+}$, but not in the *chp1*Δ*C* background (Figure 4). These data suggest that Chp1's PIN domain is required for post-transcriptional processing of subtelomeric transcripts.

Chp1's PIN domain does not possess nuclease activity

As mentioned, PIN domains are often associated with nuclease activity. We wondered whether the defect in *tlh* transcript regulation could be attributed to a possible nucleolytic activity of the PIN domain. Structure based alignment (Figure 2c) indicates that the Chp1 PIN domain has only a subset of the conserved aspartate residues critical for nuclease

activity attributed to some PIN domains. Nonetheless, Chp1 still possesses a number of amino acids in the active site that might be able to bind the Mg^{2+} ions required for nuclease function (Figure 2b). We therefore performed nuclease assays with the construct used for crystallization, but did not detect any discernible nuclease activity (data not shown), as is the case for SMG5[31].

To further test a possible nuclease activity *in vivo*, a presumed catalytically dead mutation (D904A) was engineered into the PIN domain of *chp1*$^{+}$ in an otherwise wild-type strain. In contrast to *chp1ΔC*, this mutant showed no accumulation of subtelomeric transcripts (Figure 3e, Supplementary Figure 9), consistent with our observation that the PIN domain does not possess nucleolytic activity *in vitro*.

Chp1's basic patch affects *tlh* silencing

A prominent positively charged patch is nestled between domain II and the PIN domain of Chp1 (Figure 1c and Supplementary Figure 10). In order to gauge its functional significance, we mutated two PIN-domain arginines, Arg923 and Arg924, which are part of this basic patch and are conserved (see below), to alanine. Interestingly, mutation of both arginines caused a similar defect in *tlh* transcript regulation as deletion of Chp1's entire PIN domain (Figure 3b,f,g and Supplementary Figure 9).

Chp1 is a member of a large family of fungal proteins

A PSI-Blast search using the sequence corresponding to the Chp1 fragment observed in the crystal structure identified a protein family particular to fungi (Figure 5a). Based on the sequence alignment to Chp1, the members of this family share common features, such as a chromodomain at the N terminus and a C-terminal PIN domain, separated by a distance of around 700-1000 amino acids. Within the PIN domain we observe strong conservation of the functionally important “basic patch” arginines Arg923 and Arg924. Interestingly, the Tas3 interacting residues of Chp1 are not well conserved (Figure 5b), and we were unable to identify Tas3 homologs outside the *Schizosaccharomyces* clade. It remains to be tested whether the RITS complex is conserved in fungi.

Discussion

The RITS complex plays a central role in the formation of heterochromatin.It acts as a switch that integrates the sequence specificity of the RNAi effector protein Argonaute with sensing the H3K9 methyl marks on chromatin through its chromodomain protein Chp1[13]. Once recruited to a specific location, it serves as a platform for binding of ClrC and RDRP, which lead to robust heterochromatin formation and gene silencing. The structure of Chp1 bound to Tas3 presented here shows that these two RITS subunits interact tightly to form a solid bridge between chromatin substrate and the RNAi machinery. This structure together with the structures of the Chp1 chromodomain[13] - the attachment point to chromatin, and the α-helical motif on the C terminus of Tas3[20] - a multimerization module, most likely represent most, if not all, of the structured domains of the Chp1-Tas3 complex. Long, unstructured regions connect these domains, allowing the Chp1-Tas3 backbone to span great distances between attachment sites, thereby possibly crosslinking neighboring strands of

chromatin fibers. Combining all the available information suggests cooperation between Ago1 and the Chp1-Tas3 complex in spreading on chromatin, thereby setting up a robust, polymeric platform that supports the enforcement of heterochromatin (Figure 6).

In addition to the role of the RITS complex in both RNAi and Clr4-mediated repression of transcription, the identification of a PIN domain in Chp1 provides important clues for the unique function of the Chp1-Tas3 complex in RNAi-independent silencing of telomeric transcripts. Although the PIN domain of Chp1 itself does not appear to possess endonuclease activity, the elevation of transcripts without change in Pol II occupancy in Chp1 mutants is clear evidence for an important role of Chp1 and Tas3 in post-transcriptional gene silencing (PTGS) at the subtelomere, much of it mediated by the PIN domain. The PIN domain may be involved in recruiting nuclease activities to subtelomeric regions, much like the PIN domain of the exosome subunit Rrp44/Dis3 was demonstrated to tether Rrp44 to the rest of the core exosome[30,39]. In addition, it has been previously reported that subtelomeric *tlh* transcripts are regulated by the *S. pombe* TRAMP complex and the exosome[6]. It will be interesting to determine whether the PIN domain of Chp1 is similarly involved in recruitment of the exosome to subtelomeric regions for processing of *tlh* transcripts. Since mutation of the basic region at the neck of the PIN domain yields the same loss of subtelomeric transcript regulation, it is possible that the basic region is mediating protein-protein interactions. Or it might be involved in nucleic acid binding such as binding of the *tlh* transcripts themselves, thus presenting substrates to an RNA degradation pathway, the exosome, for example, that is recruited through the PIN domain. Given that Chp1 localizes to all sites of heterochromatin, and that the exosome is also involved in regulation of centromeric transcripts[6], the question still remains as to why Chp1's PIN domain plays no role in the regulation of centromeric transcripts. One possibility is that Ago1 and the RNAi pathway dominate PTGS at the centromere, thereby masking the activity of the PIN domain. The picture thus emerging suggests that the various modules of the RITS complex play context-specific roles (Figure 6). It would be of great interest to elucidate how the various functions of RITS are specifically regulated.

It is curious that fungal EST1 proteins, in contrast to their metazoan counterparts, do not feature a PIN domain. Since we present evidence for a subtelomeric silencing role of Chp1's PIN domain, there exists a possibility that Chp1 provides a functionality that has been incorporated into the telomeric EST1 proteins in higher organisms. It is intriguing that proteins bearing both a chromodomain and a PIN domain are conserved only within fungi, and that *tlh* genes (as their name implies) are restricted to subtelomeric domains in fungi[43]. Future experiments will address whether the physical link between RNAi and heterochromatin observed in the Chp1-Tas3 structure, as well as the RNAi-independent role of Chp1's PIN domain in processing *tlh* transcripts is conserved amongst organisms of the fungal kingdom.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Chandni Ashar and Aaron Kosinski for technical support and Olivia George and Brandon Lowe for assistance, A. Héroux for help at the National Synchrotron Light Source, which is supported by Department of Energy, Office of Basic Energy Sciences. T.S. was supported by a Human Frontiers in Research Program fellowship. This work was supported by NIH grant R01GM084045, NCI Cancer Center support grant P30CA021765, the American Lebanese Syrian Associated Charities (to J.F.P.) and the Louis Morin Charitable Trust (to L.J). L.J. is an investigator of the Howard Hughes Medical Institute. We thank G.Thon, T.Nakamura, R. Martienssen and M. Zaratiegui for helpful discussions, and G. Thon, and R. Allshire for strains, and K. Gull for anti-TAT1 antibody.

References

1. Moazed D. Small RNAs in transcriptional gene silencing and genome defence. Nature. 2009; 457:413–420. [PubMed: 19158787]
2. Volpe T, Martienssen RA. RNA Interference and Heterochromatin Assembly. Cold Spring Harb Perspect Biol. 2011 Published in Advance.
3. Volpe TA, et al. Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. Science. 2002; 297:1833–1837. [PubMed: 12193640]
4. Sugiyama T, Cam H, Verdel A, Moazed D, Grewal SIS. RNA-dependent RNA polymerase is an essential component of a self-enforcing loop coupling heterochromatin assembly to siRNA production. Proc Natl Acad Sci. 2005; 102:152–157. [PubMed: 15615848]
5. Buhler M, Verdel A, Moazed D. Tethering RITS to a Nascent Transcript Initiates RNAi- and Heterochromatin-Dependent Gene Silencing. Cell. 2006; 125:873–886. [PubMed: 16751098]
6. Buhler M, Haas W, Gygi SP, Moazed D. RNAi-Dependent and -Independent RNA Turnover Mechanisms Contribute to Heterochromatic Gene Silencing. Cell. 2007; 129:707–721. [PubMed: 17512405]
7. Zhang K, Mosch K, Fischle W, Grewal SIS. Roles of the Clr4 methyltransferase complex in nucleation, spreading and maintenance of heterochromatin. Nat Struct Mol Biol. 2008; 15:381–388. [PubMed: 18345014]
8. Bayne EH, et al. Stc1: A Critical Link between RNAi and Chromatin Modification Required for Heterochromatin Integrity. Cell. 2010; 140:666–677. [PubMed: 20211136]
9. Gerace EL, Halic M, Moazed D. The Methyltransferase Activity of Clr4Suv39h Triggers RNAi Independently of Histone H3K9 Methylation. Mol Cell. 2010; 39:360–372. [PubMed: 20705239]
10. Grewal SIS, Jia S. Heterochromatin revisited. Nat Rev Genet. 2007; 8:35–46. [PubMed: 17173056]
11. Verdel A, et al. RNAi-mediated targeting of heterochromatin by the RITS complex. Science. 2004; 303:672–676. [PubMed: 14704433]
12. Creamer KM, Partridge JF. RITS—connecting transcription, RNA interference, and heterochromatin assembly in fission yeast. WIREs RNA. 2011; 2:632–646. [PubMed: 21823226]
13. Schalch T, et al. High-Affinity Binding of Chp1 Chromodomain to K9 Methylated Histone H3 Is Required to Establish Centromeric Heterochromatin. Molecular Cell. 2009; 34:36–46. [PubMed: 19362535]
14. Irvine DV, et al. Argonaute Slicing Is Required for Heterochromatic Silencing and Spreading. Science. 2006; 313:1134–1137. [PubMed: 16931764]
15. Buker SM, et al. Two different Argonaute complexes are required for siRNA generation and heterochromatin assembly in fission yeast. Nat Struct Mol Biol. 2007; 14:200–207. [PubMed: 17310250]
16. Petrie VJ, Wuitschick JD, Givens CD, Kosinski AM, Partridge JF. RNA interference (RNAi)-dependent and RNAi-independent association of the Chp1 chromodomain protein with distinct heterochromatic loci in fission yeast. Mol Cell Biol. 2005; 25:2331–2346. [PubMed: 15743828]
17. Partridge JF, et al. Functional Separation of the Requirements for Establishment and Maintenance of Centromeric Heterochromatin. Mol Cell. 2007; 26:593–602. [PubMed: 17531816]

18. Till S, et al. A conserved motif in Argonaute-interacting proteins mediates functional interactions through the Argonaute PIWI domain. Nat Struct Mol Biol. 2007; 14:897–903. [PubMed: 17891150]
19. DeBeauchamp JL, et al. Chp1-Tas3 Interaction Is Required To Recruit RITS to Fission Yeast Centromeres and for Maintenance of Centromeric Heterochromatin. Mol Cell Biol. 2008; 28:2154–2166. [PubMed: 18212052]
20. Li H, et al. An Alpha Motif at Tas3 C Terminus Mediates RITS cis Spreading and Promotes Heterochromatic Gene Silencing. Mol Cell. 2009; 34:155–167. [PubMed: 19394293]
21. Motamedi MR, et al. Two RNAi complexes, RITS and RDRC, physically interact and localize to noncoding centromeric RNAs. Cell. 2004; 119:789–802. [PubMed: 15607976]
22. Doe C, et al. The fission yeast chromo domain encoding gene chp1(+) is required for chromosome segregation and shows a genetic interaction with alpha- tubulin. Nucl Acids Res. 1998; 26:4222–4229. [PubMed: 9722643]
23. Partridge JF, Borgstrom B, Allshire RC. Distinct protein interaction domains and protein spreading in a complex centromere. Genes Dev. 2000; 14:783–791. [PubMed: 10766735]
24. Fitzgerald DJ, et al. Protein complex expression by using multigene baculoviral vectors. Nat Meth. 2006; 3:1021–1032.
25. Ariyoshi M, Schwabe JWR. A conserved structural motif reveals the essential transcriptional repression function of Spen proteins and their role in developmental signaling. Genes Dev. 2003; 17:1909–1920. [PubMed: 12897056]
26. Chang JF, et al. Structure of the coiled-coil dimerization motif of Sir4 and its interaction with Sir3. Structure. 2003; 11:637–649. [PubMed: 12791253]
27. Krissinel E, Henrick K. Inference of Macromolecular Assemblies from Crystalline State. J Mol Biol. 2007; 372:774–797. [PubMed: 17681537]
28. Clissold PM, Ponting CP. PIN domains in nonsense-mediated mRNA decay and RNAi. Curr Biology. 2000; 10:R888–R890.
29. Lebreton A, Tomecki R, Dziembowski A, Seraphin B. Endonucleolytic RNA cleavage by a eukaryotic exosome. Nature. 2008; 456:993–996. [PubMed: 19060886]
30. Bonneau F, Basquin J, Ebert J, Lorentzen E, Conti E. The Yeast Exosome Functions as a Macromolecular Cage to Channel RNA Substrates for Degradation. Cell. 2009; 139:547–559. [PubMed: 19879841]
31. Glavan F, Behm-Ansmant I, Izaurralde E, Conti E. Structures of the PIN domains of SMG6 and SMG5 reveal a nuclease within the mRNA surveillance complex. EMBO J. 2006; 25:5117–5125. [PubMed: 17053788]
32. Hodgkin J, Papp A, Pulak R, Ambros V, Anderson P. A new kind of informational suppression in the nematode Caenorhabditis elegans. Genetics. 1989; 123:301–313. [PubMed: 2583479]
33. Pulak R, Anderson P. mRNA surveillance by the Caenorhabditis elegans smg genes. Genes Dev. 1993; 7:1885–1897. [PubMed: 8104846]
34. Gatfield D, Unterholzner L, Ciccarelli FD, Bork P, Izaurralde E. Nonsense-mediated mRNA decay in Drosophila:at the intersection of the yeast and mammalian pathways. EMBO J. 2003; 22:3960–3970. [PubMed: 12881430]
35. Reichenbach P, et al. A Human Homolog of Yeast Est1 Associates with Telomerase and Uncaps Chromosome Ends When Overexpressed. Current Biology. 2003; 13:568–574. [PubMed: 12676087]
36. Snow BE, et al. Functional Conservation of the Telomerase Protein Est1p in Humans. Current Biology. 2003; 13:698–704. [PubMed: 12699629]
37. Page MF, Carr B, Anders KR, Grimson A, Anderson P. SMG-2 Is a Phosphorylated Protein Required for mRNA Surveillance in Caenorhabditis elegans and Related to Upf1p of Yeast. Mol Cell Biol. 1999; 19:5943–5951. [PubMed: 10454541]
38. Ohnishi T, et al. Phosphorylation of hUPF1 induces formation of mRNA surveillance complexes containing hSMG-5 and hSMG-7. Mol Cell. 2003; 12:1187–1200. [PubMed: 14636577]
39. Schneider C, Leung E, Brown J, Tollervey D. The N-terminal PIN domain of the exosome subunit Rrp44 harbors endonuclease activity and tethers Rrp44 to the yeast core exosome. Nucl Acids Res. 2009; 37:1127–1140. [PubMed: 19129231]

40. Hansen KR, Ibarra PT, Thon G. Evolutionary-conserved telomere-linked helicase genes of fission yeast are repressed by silencing factors, RNAi components and the telomere-binding protein Taz1. Nucl Acids Res. 2006; 34:78–88. [PubMed: 16407326]
41. Sadaie M, Iida T, Urano T, Nakayama Jichi. A chromodomain protein, Chp1, is required for the establishment of heterochromatin in fission yeast. EMBO J. 2004; 23:3825–3835. [PubMed: 15372076]
42. Sugiyama T, et al. SHREC, an Effector Complex for Heterochromatic Transcriptional Silencing. Cell. 2007; 128:491–504. [PubMed: 17289569]
43. Rehmeyer CJ, Li W, Kusaba M, Farman ML. The telomere-linked helicase (TLH) gene family in Magnaporthe oryzae: revised gene structure reveals a novel TLH-specific protein motif. Curr Genet. 2009; 55:253–262. [PubMed: 19360408]
44. Ashkenazy H, Erez E, Martz E, Pupko T, Ben-Tal N. ConSurf 2010: calculating evolutionary conservation in sequence and structure of proteins and nucleic acids. Nucl Acids Res. 2010; 38:W529–W533. [PubMed: 20478830]
45. Luft JR, DeTitta GT. A method to produce microseed stock for use in the crystallization of biological macromolecules. Acta Cryst D. 1999; 55:988–993. [PubMed: 10216295]
46. Kabsch W. XDS. Acta Cryst D. 2010; 66:125–132. [PubMed: 20124692]
47. Pape T, Schneider TR. HKL2MAP: a graphical user interface for macromolecular phasing with SHELX programs. J Appl Cryst. 2004; 37:843–844.
48. Cowtan K. “dm”: An automated procedure for phase improvement by density modification. Joint CCP4 and ESF-EACBM Newsletter on Protein Crystallography. 1994; 31:34–38.
49. Emsley P, Cowtan K. Coot: model-building tools for molecular graphics. Acta Crystallogr D Biol Crystallogr. 2004; 60:2126–2132. [PubMed: 15572765]
50. Adams PD, et al. PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Cryst D. 2010; 66:213–221. [PubMed: 20124702]
51. Baker NA, Sept D, Joseph S, Holst MJ, McCammon JA. Electrostatics of nanosystems: Application to microtubules and the ribosome. Proc Natl Acad Sci. 2001; 98:10037–10041. [PubMed: 11517324]
52. Holm L, Rosenstrom P. Dali server: conservation mapping in 3D. Nucl Acids Res. 2010; 38:W545–W549. [PubMed: 20457744]
53. Krissinel E, Henrick K. Secondary-structure matching (SSM), a new tool for fast protein structure alignment in three dimensions. Acta Crystallogr D Biol Crystallogr. 2004; 60:2256–2268. [PubMed: 15572779]
54. Thon G, Verhein-Hansen J. Four Chromo-domain proteins of Schizosaccharomyces pombe differentially repress transcription at various chromosomal locations. Genetics. 2000; 155:551–568. [PubMed: 10835380]
55. Woods A, et al. Definition of individual components within the cytoskeleton of Trypanosoma brucei by a library of monoclonal antibodies. J Cell Sci. 1989; 93(Pt 3):491–500. [PubMed: 2606940]
56. Shanker S, et al. Continuous Requirement for the Clr4 Complex But Not RNAi for Centromeric Heterochromatin Assembly in Fission Yeast Harboring a Disrupted RITS Complex. PLoS Genet. 2010; 6:e1001174. [PubMed: 21060862]

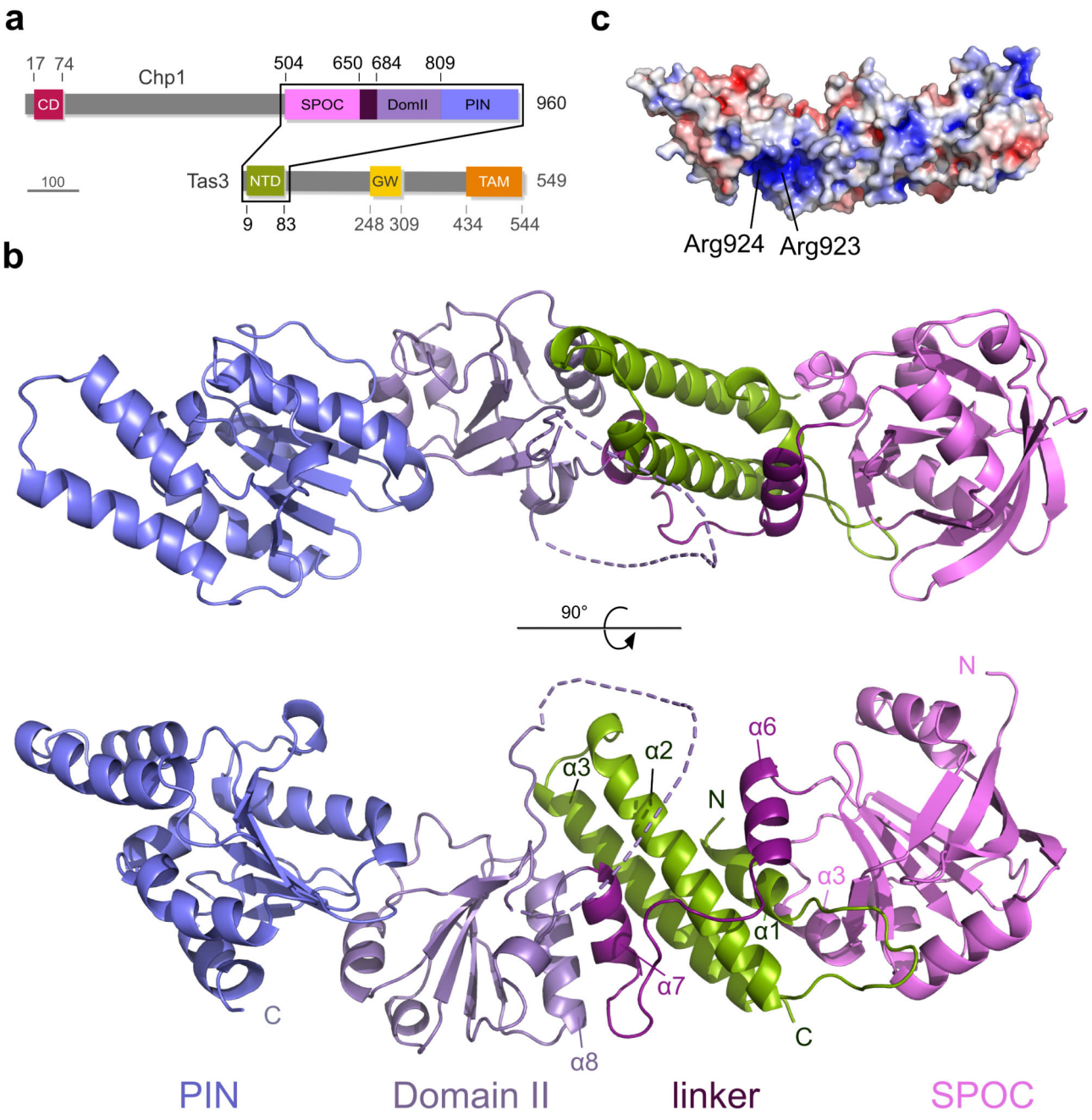

Figure 1. Structure of the Chp1-Tas3 complex

(a) Domain representation of Chp1 and Tas3 depicting the C-terminal domain (CTD) of Chp1 defined by limited proteolysis and the N-terminal domain (NTD) of Tas3. Also indicated are the chromodomain (CD) of Chp1, the GW domain and the C-terminal Tas3 α-helical motif (TAM). Numbers correspond to amino acid positions. The boxed region indicates the regions corresponding to the crystal structure. **(b)** Cartoon representation of the of the Chp1-Tas3 interaction interface as observed in the crystal structure (chains A and B without the OSS tag, loops not observed in electron density are indicated by dashed lines). Chp1 SPOC domain (domain 1, aa 515-651) is colored pink, Chp1 linker (aa 652-683)

purple, domain II (aa 684-808) lilac, and PIN domain (aa 809-960) blue. Tas3 NTD is green. The bottom panel resembles the hull of a ship. **(c)** Electrostatic potential mapped onto solvent accessible surface of the Chp1-Tas3 complex (contoured from -5 to +5 eV). Orientation of the molecule corresponds to the view shown in the bottom of panel b.

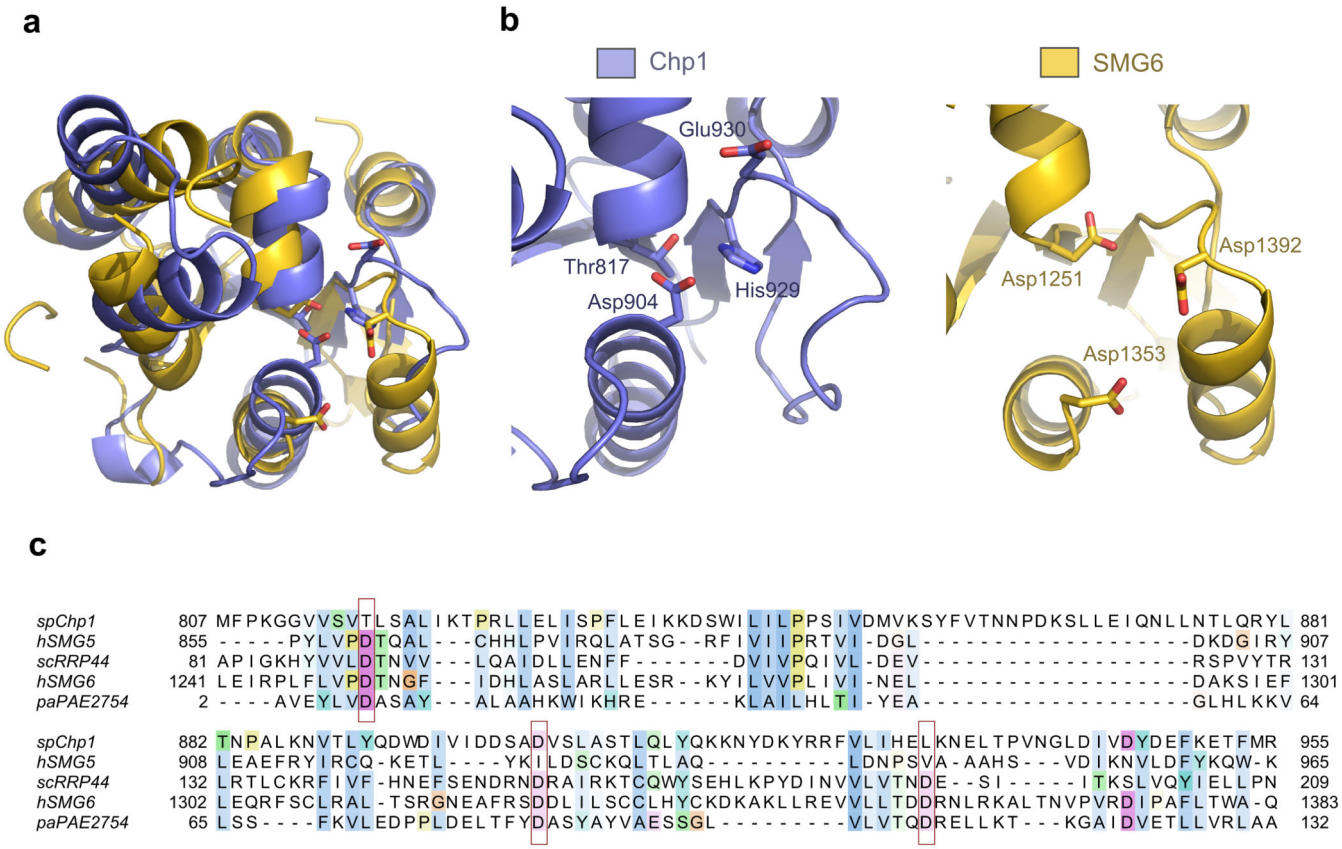

Figure 2. The Chp1 PIN domain

(a) Superposition of Chp1 PIN domain (blue) on the PIN domain of SMG6 (gold). **(b)** Comparison of SMG6 active site with corresponding residues in the PIN domain of Chp1. **(c)** Structure based sequence alignment of PIN domains from *S. pombe* Chp1, human SMG5 and SMG6, as well as the nuclease *P. areo* PAE2754. Red boxes indicate the nuclease active site residues. Gaps in Chp1 are not shown.

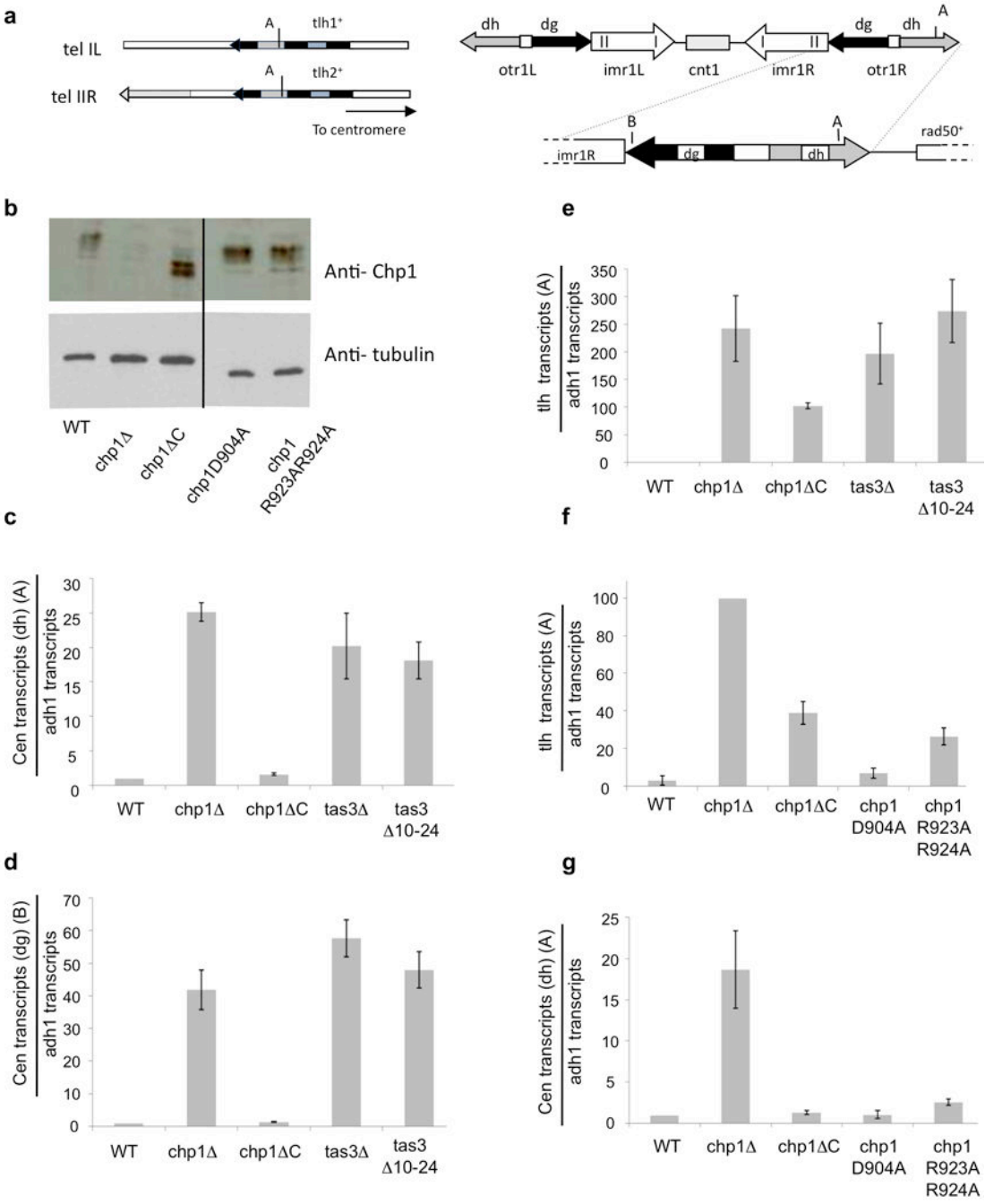

Figure 3. Chp1's PIN domain regulates subtelomeric, but not centromeric transcript accumulation

(a) Cartoon of heterochromatic loci analyzed. Subtelomeric transcripts were monitored from helicase region (A) of *tlh* genes located on chromosomes 1 and 2 and not from region of centromeric repeat homology (crosshatched). Centromeric transcripts were measured from both *dh* (A) and *dg* (B) repeats of centromeres. **(b)** Real time PCR measurements of *tlh* transcripts relative to *adh1* euchromatic control reveal accumulation of subtelomeric transcripts in cells lacking the Chp1 PIN domain (*chp1ΔC*). **(c, d)** Centromeric transcripts do

not accumulate in *chp1*Δ*C* cells. **(e)** Putative enzymatic mutant of Chp1 PIN domain, *chp1D904A*, shows no disruption of subtelomeric silencing, but mutation of the "basic patch" residues *chp1R923A,R924A* causes similar accumulation of *tlh* transcripts to the *chp1*Δ*C* mutant. Samples were normalized to *chp1*Δ as 100%. **(f)** Only *chp1* deletion, but not point mutation or removal of the PIN domain promotes centromeric transcript accumulation. Values were normalized to WT. **(g)** Western analysis of *chp1*Δ*C* and *chp1* point mutants shows that all proteins are stably expressed when compared to tubulin levels revealed by reprobing the same immunoblot. Black line marks where the immunoblot was cut to remove additional samples. Strains analyzed: **b–d**: PY42,90,[5368,5369],938,3506, **e–f**: PY42, 90, 5368, [5684, 5884], [5679, 5680] and **g**: PY42, 90, 5368, 5684, 5679. Error bars represent SEM of 2–5 biological replicates.

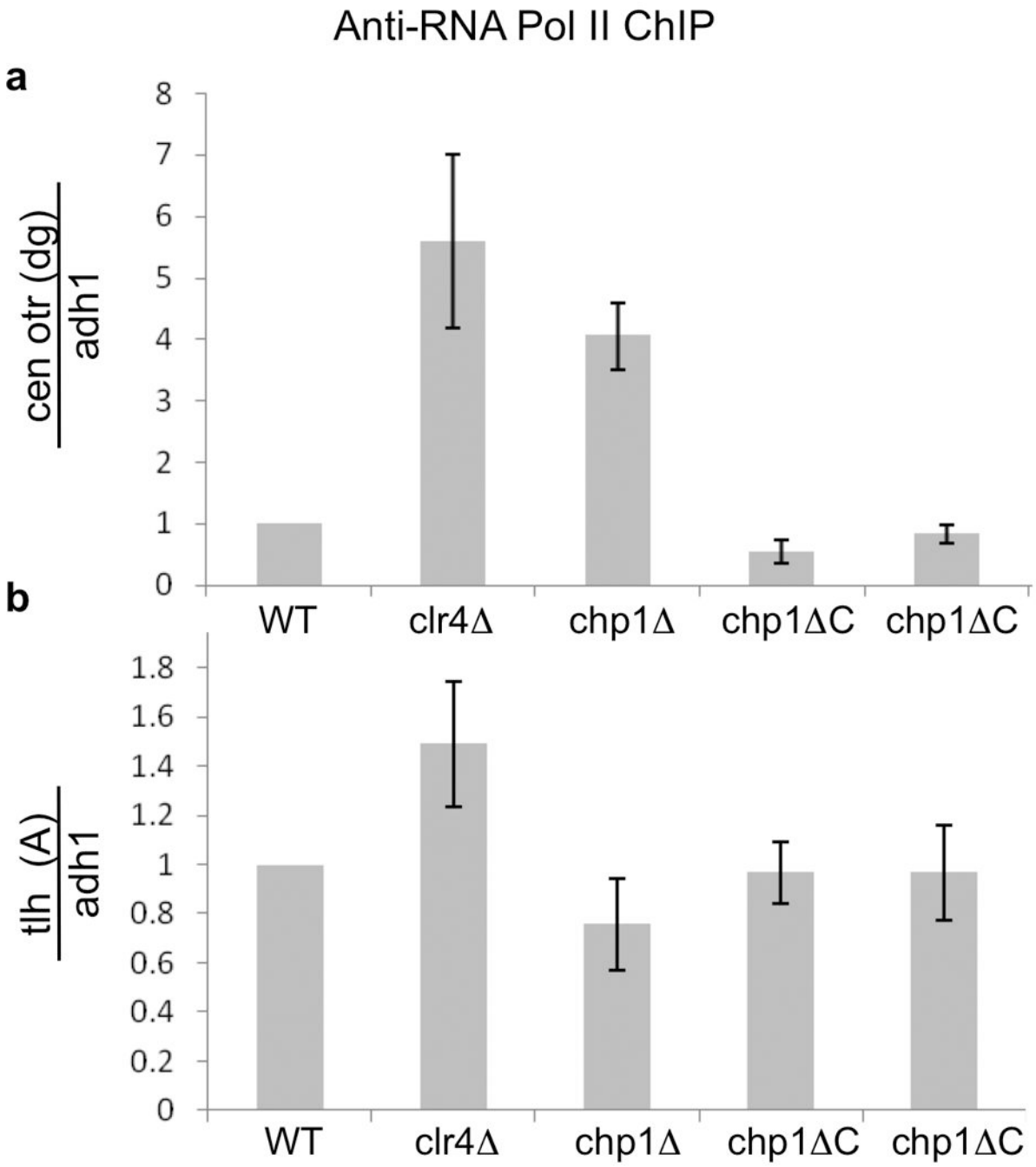

Figure 4. Chp1's PIN domain promotes post-transcriptional regulation of *tlh* transcripts

(a) ChIP analysis with anti-RNA Pol II antibodies demonstrates that Clr4 and Chp1, but not the Chp1 PIN domain, prevent RNA Pol II access to centromeric sequences. (**b**) In contrast, access of RNA Pol II to subtelomeric sequences is not influenced by Chp1 or the Chp1 PIN domain, and is consistent with a post-transcriptional role for the PIN domain in *tlh* regulation. Similar data were obtained for Pol II ChiP at the *tlh* promoter region. Strains analyzed: PY 42, 1798, 5802, 5368 and 5369. Error bars represent SEM of 2 independent ChIP experiments.

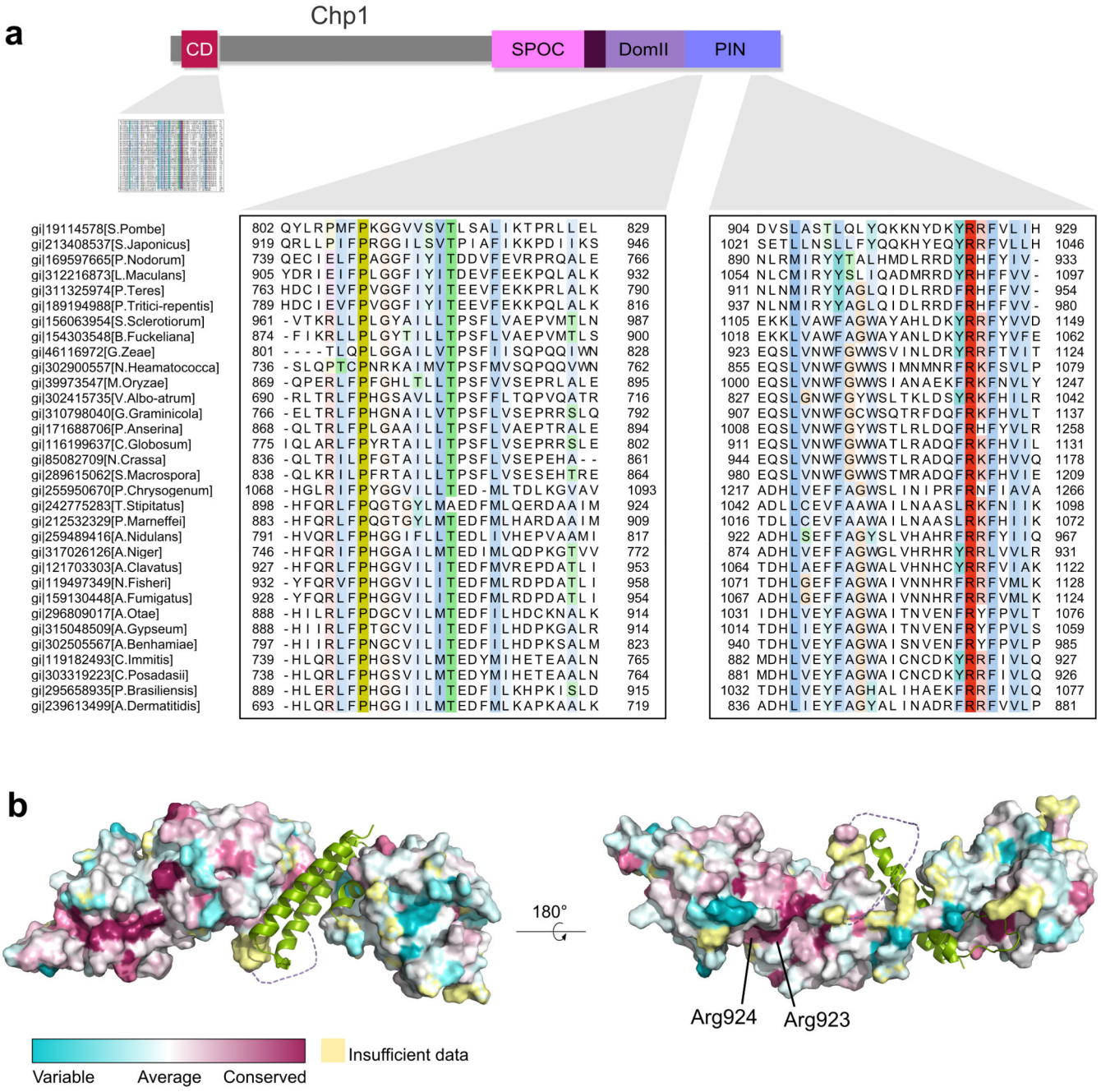

Figure 5. Family of Chp1 proteins

(a) Surface representation of the Chp1-Tas3 structure colored according to conservation scores computed from an alignment of the Chp1 family members by ConSurf[44]. (**b**) Schematic domain diagram with selected alignment blocks shown for the regions indicated. Chp1 orthologs (Table S1) were identified by PSI-Blast using Chp1 residues 504–960 and aligned using MAFFT. ClustalX colors are shown for highly conserved residues.

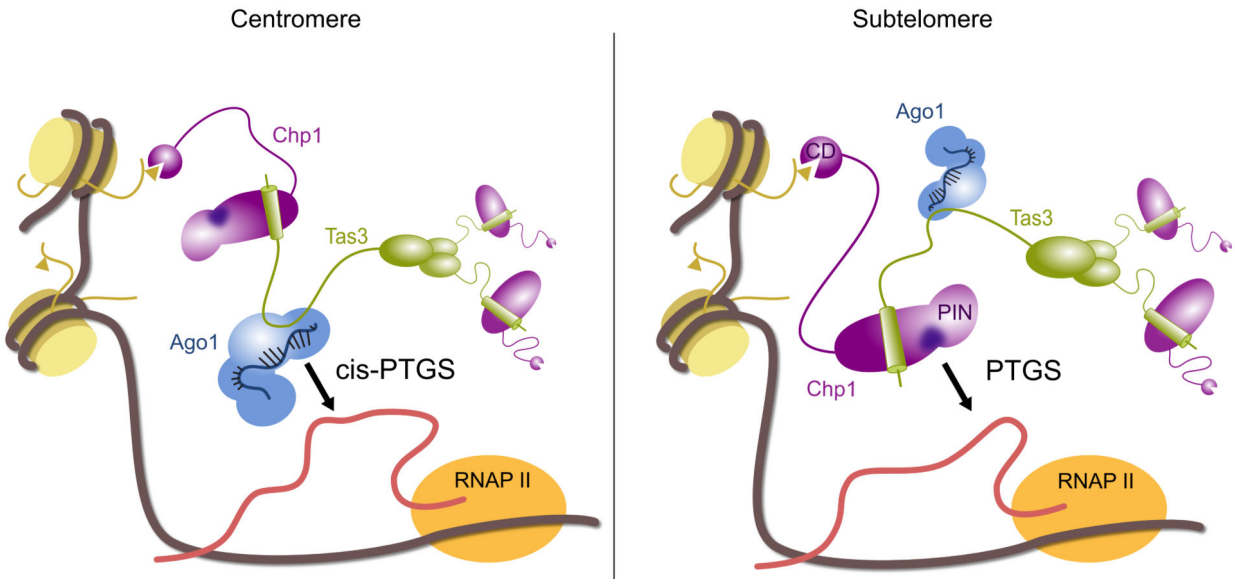

Figure 6. Context-specific functions of RITS subunits at centromeres and subtelomeres

The schematic representation of the RITS complex integrates our current understanding of the complex as a collection of stable modules tethered together by long flexible linkers. The chromodomain (CD) of Chp1 anchors RITS to H3K9 methyl marks (triangle) on nucleosomes with the Chp1-Tas3 interface forming a solid bridge between chromatin and the RNAi machinery. The GW domain of Tas3 binds to Ago1 and the C-terminal Tas3 α-helical motif promotes oligomerization and spreading. At the centromere, cis-restricted post-transcriptional gene silencing (cis-PTGS) through RITS is dominated by Ago1 activity and the RNAi machinery. In contrast, at the subtelomeric regions RITS exerts its function in PTGS mainly through Chp1 and Tas3 with a significant contribution by the PIN domain. An RNA transcript is shown in red and the siRNA is shown in blue.

Table 1

Data collection and refinement statistics

	Native	SeMet
Data collection		
Space group	C2221	C2221
Cell dimensions		
a, b, c (Å)	104.88, 172.20, 198.68	104.92, 172.78, 198.91
α β γ (°)	90, 90, 90	90, 90, 90
Wavelength (Å)	0.9792	0.9792
Resolution (Å)*	89.7–2.90 (2.98–2.90)	89.7–2.90 (2.98–2.90)
R_{sym} or R_{merge} (%)*	6.9 (48.0)	6.3 (46.0)
I/σ(I)*	8.04 (1.78)	15.45 (2.94)
Completeness (%)*	99.7 (99.8)	100 (100)
Redundancy*	1.93 (1.93)	3.8 (3.86)
Refinement		
Resolution (Å)*	65.1–2.9 (2.97–2.9)	
No. reflections*	40128 (2712)	
R_{work} / R_{free}(%)*	21.23 (30.7)/24.35 (38.5)	
No. atoms (non-hydrogen)	9309	
Protein	9272	
Ions	11	
Water	26	
B-factors		
Protein	90.3	
Ions	78.7	
Water	48.5	
r.m.s deviations		
Bond lengths (Å)	0.006	
Bond angles (°)	0.541	

*Values in parentheses are for highest-resolution shell.