⌘ *Author's Choice*

Identification of Noncoding Transcripts from within CENP-A Chromatin at Fission Yeast Centromeres*⬚

Eun Shik Choi[‡]**, Annelie Strålfors**[§]**, Araceli G. Castillo**[‡1]**, Mickaël Durand-Dubief**[§]**, Karl Ekwall**[§2]**, and Robin C. Allshire**[‡3]

From the ‡*Wellcome Trust Centre for Cell Biology and Institute of Cell Biology, The University of Edinburgh, Edinburgh EH9 3JR, Scotland, United Kingdom and the* §*Department of Biosciences and Nutrition, Center for Biosciences, Karolinska Institutet, NOVUM, 141 57 Huddinge, Sweden*

The histone H3 variant CENP-A is the most favored candidate for an epigenetic mark that specifies the centromere. In fission yeast, adjacent heterochromatin can direct CENP-A^{Cnp1} chromatin establishment, but the underlying features governing where CENP-A^{Cnp1} chromatin assembles are unknown. We show that, in addition to centromeric regions, a low level of CENP-A^{Cnp1} associates with gene promoters where histone H3 is depleted by the activity of the Hrp1^{Chd1} chromatin-remodeling factor. Moreover, we demonstrate that noncoding RNAs are transcribed by RNA polymerase II (RNAPII) from CENP-A^{Cnp1} chromatin at centromeres. These analyses reveal a similarity between centromeres and a subset of RNA-PII genes and suggest a role for remodeling at RNAPII promoters within centromeres that influences the replacement of histone H3 with CENP-A^{Cnp1}.

Centromeres are the specific chromosomal loci at which kinetochore assembly occurs. Extensive investigation of centromeric DNA from a wide variety of eukaryotic cells indicates that the primary sequence is not conserved. Despite this, all functional centromeres, including neocentromeres formed at ectopic chromosomal loci, share a unique chromatin composition in which the evolutionarily conserved histone H3 variant CENP-A replaces canonical histone H3. At many centromeres, this CENP-A kinetochore chromatin is formed on repetitive arrays such as α-satellite on human chromosomes. However, the fact that this CENP-A chromatin is flanked by similar repeats assembled in heterochromatin (1–3) makes it difficult to distinguish repeats coated in heterochromatin from those assembled in CENP-A chromatin. In the fission yeast

Schizosaccharomyces pombe, the heterochromatic outer repeats are distinct from the central domain over which CENP-A^{Cnp1} and the kinetochore assemble (1). Although heterochromatin is required for the *de novo* assembly of CENP-A^{Cnp1} chromatin on central domain DNA, it is dispensable for the subsequent maintenance of CENP-A^{Cnp1} chromatin (4). Thus, kinetochore-associated DNAs may possess unidentified features that are not apparent from the primary sequences that make them favorable substrates for CENP-A^{Cnp1} deposition and kinetochore assembly. Previous analyses have suggested that the acetylated state of histones may influence CENP-A assembly (5, 6). Moreover, transcripts homologous to centromere-associated DNAs have been detected in various organisms, and retrotransposon RNAs are implicated in centromere chromatin structure (7–10). In fission yeast, it has been shown that a GATA-like transcription factor is required for efficient CENP-A^{Cnp1} deposition (11). Also in fission yeast, the ATP-dependent remodeling factor Hrp1 (orthologous to *Saccharomyces cerevisiae* Chd1 (<u>c</u>hromo-<u>h</u>elicase <u>D</u>NA-binding protein <u>1</u>)) affects CENP-A^{Cnp1} deposition (12). Chd1 is involved in transcriptional elongation by RNA polymerase II (RNAPII)[4] (13) and has been shown to facilitate replication-independent histone H3 exchange (14). An attractive hypothesis is that transcription underlies chromatin remodeling in the centromeric DNAs, which in turn promotes CENP-A^{Cnp1} deposition. Here, we investigated this possibility.

EXPERIMENTAL PROCEDURES

Standard procedures were used for growth and genetic manipulation. The details of PCR primers are listed in supplemental Table 1. The *S. pombe* strains used in this study are listed in supplemental Table 2. The procedures used are described under supplemental "Methods."

ChIP—Cells were fixed with 1% paraformaldehyde and lysed by bead beating. Chromatin was solubilized by shearing with a Bioruptor sonicator and immunoprecipitated using 10 μl of anti-CENP-A^{Cnp1} antiserum with protein G-agarose beads. ChIPs were then analyzed by quantitative PCR.

ChIP-Chip—DNA was immunoprecipitated and hybridized to Affymetrix GeneChip® *S. pombe* tiling 1.0FR arrays as described previously (15). 10 μl of anti-CENP-A^{Cnp1} and 1.5 μg of anti-H3 (ab1791, ABCAM) antibodies were added to 100 μl

[4] The abbreviations used are: RNAPII, RNA polymerase II; TUK, transcript from under kinetochores; RACE, rapid amplification of cDNA ends.

of chromatin extract. Hrp1[Chd1] binding data and histone H3 density maps are from a previous study (16).

Raw data from Affymetrix (.CEL format) were analyzed with Affymetrix TAS (tiling analysis) version 1.1 software and visualized with Affymetrix IGB (integrated genome browser). The data were normalized using quantile normalization plus scaling and run with a bandwidth of 20. *p* values were calculated using hypergeometric probability distribution in R version 2.12.0.

RESULTS

Hrp1[Chd1] has previously been implicated in CENP-A[Cnp1] deposition but is not essential for cell viability (12). Expression of RNAPII-transcribed marker genes such as *arg3*⁺ is repressed when placed within the central domain, and silencing of *cen1*: *arg3*⁺ is highly sensitive to defective CENP-A[Cnp1] deposition (17); however, this silencing is only partially impaired in *hrp1Δ* cells (Fig. 1*A*). This suggests that redundant mechanisms operate to ensure CENP-A[Cnp1] deposition in the absence of Hrp1[Chd1]. In agreement with this and previous analyses (12), when *hrp1Δ* was combined with the *mis6-302* temperature-sensitive mutation (defective in CENP-A[Cnp1] deposition), it reduced the restrictive temperature of *mis6-302*. Furthermore, *hrp1Δ* also reduced the restrictive temperature of *cnp1-87*, a weak temperature-sensitive allele (Fig. 1*B*) (18). Consistent with this, the levels of CENP-A[Cnp1] associated with the central domain were further reduced in *hrp1Δ cnp1-87* double mutants compared with either single mutant (Fig. 1*C*). We conclude that Hrp1 facilitates the assembly of CENP-A[Cnp1] chromatin, and it becomes essential when Mis6 or CENP-A[Cnp1] function is impaired.

Genome-wide analyses of CENP-A[Cnp1] and histone H3 localization by ChIP on tiling arrays with anti-CENP-A[Cnp1] and anti-H3 antibodies confirmed that Hrp1[Chd1] is required to maintain normal levels of CENP-A[Cnp1] and that, in *hrp1Δ* cells, H3 levels increase across the central domain (Fig. 1*D* and supplemental Fig. S1). Previously, we have shown that Hrp1[Chd1] acts at a subset of gene promoters to disassemble histone H3-containing nucleosomes close to the transcription start sites (16). Further examination of the genome-wide analyses revealed that low but detectable levels of CENP-A[Cnp1] associate with a significant proportion of promoters in wild-type cells at which Hrp1[Chd1] acts to disassemble H3-containing nucleosomes (Fig. 2*A*). CENP-A[Cnp1] association is significantly reduced at some, but not all, of these promoters in *hrp1Δ* cells, and in agreement with this, we saw an increase in H3 ($p = 2.7e^{-4}$, hypergeometric probability) (Fig. 2, *B* and *C*). Some promoters show an increase in CENP-A[Cnp1] association and a decrease in H3 in *hrp1Δ* cells; however, this is less significant (Fig. 2*D*). These analyses imply that Hrp1[Chd1] directly participates in a remodeling process that evicts H3 and allows CENP-A[Cnp1] deposition at the promoters of some genes. However, additional factors, such as Scm3, must also contribute to the replacement of H3 with CENP-A[Cnp1] and/or CENP-A[Cnp1] maintenance at centromeres (19, 20). This bears resemblance to the transcription-coupled replacement of H3.1 with H3.3 in metazoa (21) and suggests that remodeling at some promoters of RNAPII

genes is intimately associated with the destabilization of H3-containing nucleosomes to encourage the assembly of CENP-A[Cnp1] chromatin. Nucleosome replacement in *S. cerevisiae* is more prominent at promoters than within coding regions (22), and the genome-wide effects of Hrp1[Chd1] on H3 eviction in fission yeast are more pronounced at promoters compared with coding regions and 3′-intergenic regions (16), accounting for the preferential association of CENP-A[Cnp1] with the promoters of genes at which Hrp1[Chd1] functions.

The close correlation between CENP-A[Cnp1] association and Hrp1[Chd1] function at gene promoters as well as at centromeres raises the possibility that centromeric DNA may contain promoters for hitherto unidentified transcription elements. It is well established that transcripts are produced from the heterochromatic outer repeats of fission yeast centromeres and processed by the RNAi pathway (23, 24). To determine whether the central kinetochore domain is also transcribed, RT-PCR was performed with primer pairs (PP1–PP10) complementary to centromere 1 (*cen1*) (Fig. 3*A*). PP1–PP3 detect part of the outer repeat heterochromatic transcript. PP4 flanks the tRNA[Ala]/tRNA[Glu] genes, whereas PP5–PP10 lie within the central subkinetochore domain. In wild-type cells, heterochromatic transcripts were detected in a region immediately adjacent to the outer repeats (PP1 and PP2), but transcripts were not apparent in the central domain (Fig. 3*B*). Outer repeat transcripts are known to accumulate in mutants defective in RNAi or heterochromatin integrity (23, 24). Several studies in *S. cerevisiae* indicate that aberrant or cryptic transcripts are degraded by 5′–3′- and/or 3′–5′-exoribonucleases; 3′-end processing can contribute to RNA stability (25). Indeed, in fission yeast, outer repeat heterochromatin transcript levels are also regulated by the exosome (26–28). We therefore tested whether conditional mutations in Pfs2 (*pfs2-11* temperature-sensitive; polyadenylation factor I subunit 2) (29), Dhp1 (*dhp1-1* temperature-sensitive; 5′–3′-exoribonuclease orthologous to Xrn2/Rat1) (30), or Dis3 (*dis3-54* cold-sensitive; 3′–5′-exoribonuclease component of the exosome) (27) allow accumulation of RNA homologous to the central domain. Transcripts from the central domain were clearly detected with PP5, PP6, PP7, PP9, and PP10 in *dhp1-1*, *pfs2-11*, and *dis3-54* cells, but not in wild-type cells, under restrictive conditions (36 or 18 °C) (Fig. 3*B*). We conclude that a large proportion of the central domain is transcribed but that the resulting transcripts are normally undetectable due to their rapid turnover. RT-PCR also detected transcripts homologous to the central domains of *cen2* and *cen3* in *dis3-54* cells (Fig. 3*C*). Thus, transcription of subkinetochore chromatin is a general property of the three centromeres, and these transcripts from under kinetochores (TUKs) are normally degraded by the exosome.

Northern analyses with an RNA probe specific for the *cen1* central domain confirmed the presence of TUKs; no obvious signal was observed in wild-type cells, but a smear of transcripts was detected in RNA-processing mutants (*pfs2-11*, *dhp1-1*, and *dis3-54*) (Fig. 4*A* and supplemental Fig. S2). We also examined the temperature-sensitive mutants *mis6-302* and *cnp1-1*,
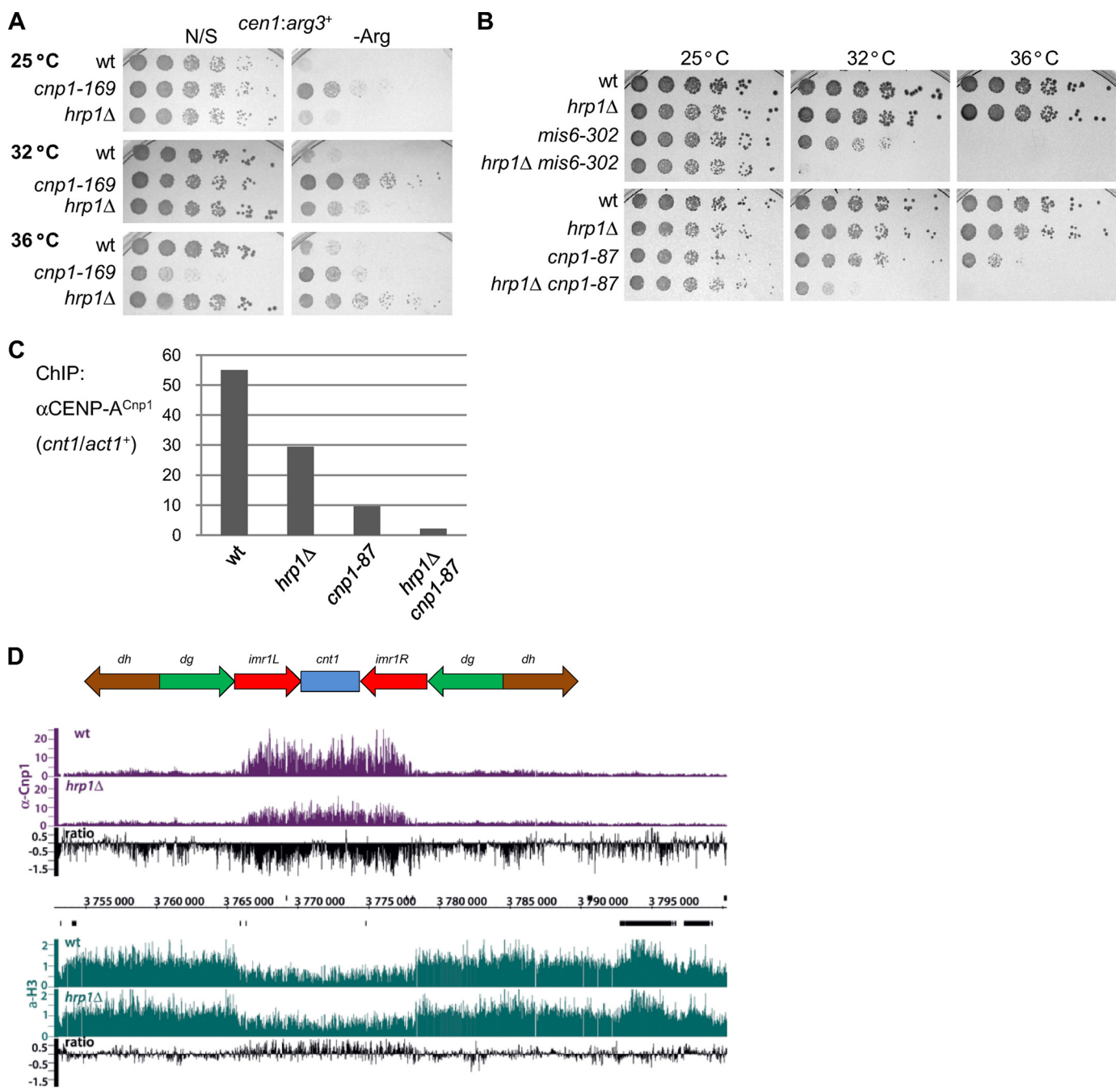
FIGURE 1. **Chromatin-remodeling factor Hrp1[Chd1] contributes to CENP-A[Cnp1] chromatin formation.** *A*, silencing of *cen1:arg3[+]* in WT, *cnp1-169*, and *hrp1Δ* cells. The growth assay was performed on non-selective (*N/S*) or arginine-depleted (*−Arg*) plates at 25, 32, and 36 °C. *B*, viability of cells bearing *hrp1Δ* combined with *mis6-302* (*upper panels*) or with *cnp1-87* (*lower panels*) compared with wild-type and single mutants grown at 25, 32, or 36 °C. *C*, CENP-A[Cnp1] ChIP analyses in WT, *hrp1Δ*, *cnp1-87*, and *hrp1Δ cnp1-87* cells grown at 36 °C. The enrichment of the *cnt1* product was compared with input DNA relative to the *act1[+]* control by quantitative PCR. *D*, genome browser view of *cen1* showing ChIP-chip binding profiles for CENP-A[Cnp1] (*purple*) and H3 (*green*) in WT and *hrp1Δ* cells (as indicated) at 30 °C. The relative ratios of CENP-A[Cnp1] and H3 (*hrp1Δ*/WT) are indicated (*black*). Data on the *y axis* are presented in log 2 scale, and the *x axis* shows genome positions in base pairs.

which have reduced levels of CENP-A[Cnp1] chromatin over the central domain (31, 32). Surprisingly, transcripts with discrete sizes (~0.5 kb) were identified in both mutants (Fig. 4*A*); these discrete transcripts (discrete TUKs) were highly enriched in poly(A)-selected RNA (Fig. 4*B*). A lower level of these specific transcripts was also enriched in the poly(A) fraction from *dis3-54* (Fig. 4*B*, *arrowhead*). Dis3 is the key catalytic subunit of the exosome required to degrade aberrant transcripts, whereas Pfs2 and Dhp1 are required for

normal 3′-end formation/termination (27, 29, 30). *pfs2-11* and *dhp1-1* may generate transcripts with extended 3′-ends due to transcriptional read-through into downstream regions, resulting in the observed heterogeneous smear of transcripts (Fig. 4, *A* and *B*). The specific increase in discrete TUKs observed in *mis6-302* and *cnp1-1* cells could be interpreted to mean that intact CENP-A[Cnp1] chromatin prevents expression of these transcripts; however, our analyses suggest that TUKs are constitutively produced and turned over
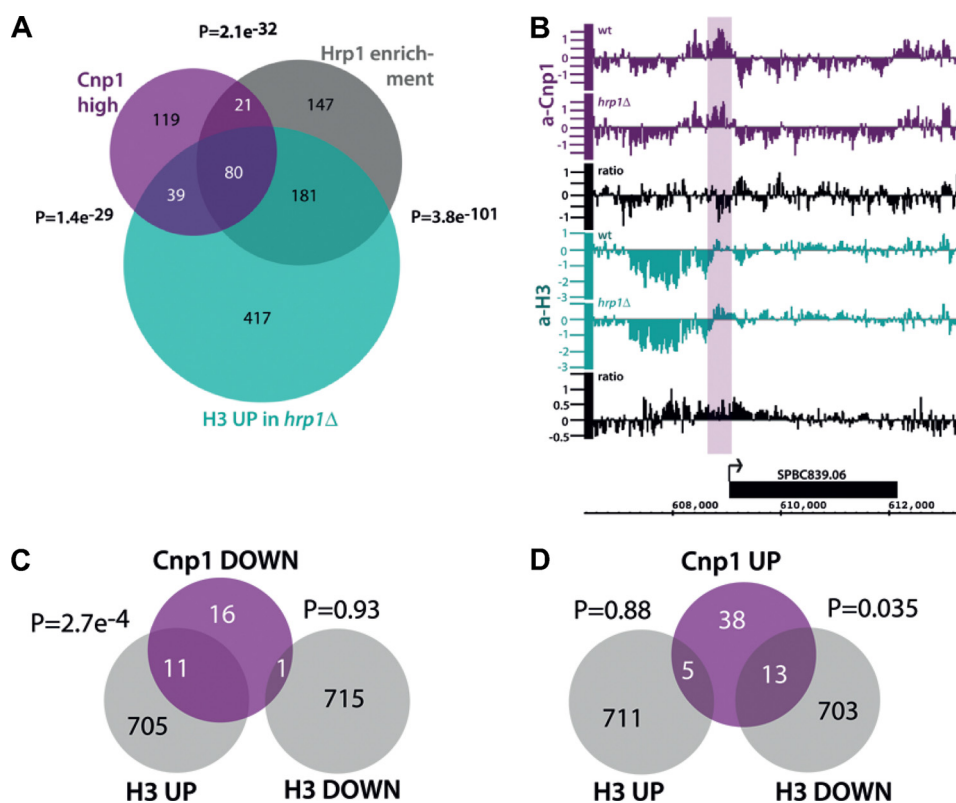
FIGURE 2. **Genome-wide CENP-A^{Cnp1} localization correlates with Hrp1^{Chd1} occupancy and H3 density changes in *hrp1Δ* cells.** *A*, Venn diagram illustrating the overlap of CENP-A^{Cnp1} enrichment with Hrp1^{Chd1} occupancy and H3 density increase in *hrp1Δ* cells (16). A cutoff of 1.5 was used for CENP-A^{Cnp1} enrichment. The *p* values indicate the probability of overlap generated by a hypergeometric probability test using R. *B*, genome browser view of gene *SPBC839.06* with reduced CENP-A^{Cnp1} (*purple*) and increased H3 (*green*) levels at its promoter in *hrp1Δ* cells. The *arrow* indicates the direction of transcription. The relative ratios of CENP-A^{Cnp1} and H3 are indicated (*black*). Data on the *y* axis are presented in log 2 scale, and the *x* axis shows genome positions in base pairs. *C*, Venn diagram showing the overlap between promoters where CENP-A^{Cnp1} is reduced, promoters where H3 is increased, and promoters where H3 is reduced in *hrp1Δ* cells. A cutoff of 1.2 was used for CENP-A^{Cnp1} reduction. *D*, Venn diagram showing the overlap between promoters where CENP-A^{Cnp1} is increased, promoters where H3 is increased, and promoters where H3 is reduced in *hrp1Δ* cells. A cutoff of 1.2 was used for the CENP-A^{Cnp1} increase.

by the exosome in wild-type cells. The chromatin context in *mis6-302* and *cnp1-1* cells is dramatically altered from CENP-A^{Cnp1} to H3 chromatin; CENP-A^{Cnp1} chromatin may interfere with events required for the formation of specific transcripts, such as accurate 3′-termination, ensuring their turnover. Using RNA probes complementary to either reverse or forward strands of the *cen1* central core (cc1/tm1) (supplemental Fig. S2), we detected other discrete transcripts in distinct central domain regions in poly(A) RNA from *cnp1-1* cells (Fig. 4, *C* and *D*). To identify the 5′-ends of these central domain noncoding transcripts, we employed 5′-rapid amplification of cDNA ends (RACE)/PCR on 5′-capped poly(A) RNA extracted from wild-type and *cnp1-1* cells. This demonstrated that these novel/unusual transcripts are produced from within the central domain and do not arise by read-through from outer repeat transcripts (Fig. 4*E* and supplemental Fig. S3). The high sensitivity of 5′-RACE/PCR allowed the detection of transcripts in wild-type cells with some primers (Fig. 4*E*, *black arrows*, and supplemental Fig. S3). The transcription start sites for these transcripts were identical in wild-type and *cnp1-1* cells, suggesting that they are indeed produced from wild-type centromeres, albeit at a lower level. The fact that these transcripts are 5′-capped and polyadenylated indicates that TUKs are produced by RNAPII. Consistent with alterations

in central domain chromatin affecting transcription (lower CENP-A^{Cnp1} and higher H3 deposition) (Fig. 1*D*), increased levels of the central domain transcript were also detectable in *hrp1Δ* cells (supplemental Fig. S4).

On the basis of the 5′-RACE analyses above, we designed improved probes for Northern analyses to detect the forward and reverse strands of the *cen1* central core (cc1/tm1) (supplemental Fig. S2) in additional mutants known to affect kinetochore integrity (*mis12-537*, *mis16-53*, and *mis18-262* in addition to *mis6-302* and *cnp1-1*) (6). At 36 °C, transcripts were detected in all mutants apart from *mis12-537* with this TM-forward[5] probe (Fig. 4*F*). Some transcripts were even detectable in *mis6-302*, *cnp1-1*, and *mis16-53* at 25 °C, the permissive temperature. In all mutants, including *mis12-537*, this TM-reverse probe allowed detection of other transcripts (Fig. 4*G*) in addition to those observed with the original probe (Fig. 4*D*). Together, these analyses indicate that cryptic transcription is prevalent in the central kinetochore domain of fission yeast centromeres and revealed only in cells defective in RNA turnover or formation of subkinetochore chromatin. In support of this, low levels of H3K4

---

[5] TM represents a 3.3-kb element shared between the central domains of *cen2* and *cen3*.
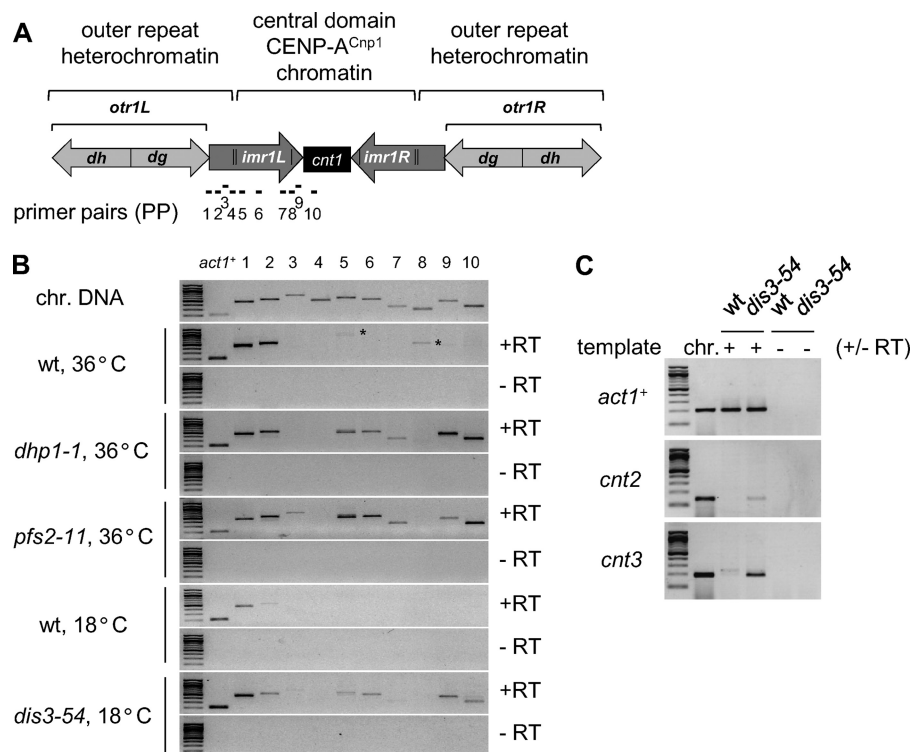
FIGURE 3. **Accumulation of central domain transcripts in RNA-processing mutants.** *A*, schematic of fission yeast *cen1*, indicating the central core (*cnt*), innermost repeat (*imr*), and outer repeats (*otr/dg-dh*). Regions amplified by primer pairs (PP1–PP10) used in RT-PCR are indicated below (*black bars*). *B*, RT-PCR analysis of transcripts from *cen1* and the *act1+* control. Wild-type, *dhp1-1*, and *pfs2-11* cells were grown at 25 °C and then shifted to 36 °C for 6 h before RNA extraction. Wild-type and *dis3-54* cells were grown at 36 °C and shifted to 18 °C for 9 h before RNA extraction. −*RT*, negative control performed without reverse transcriptase; *, unspecific products. PCR with a chromosomal DNA (*chr. DNA*) template was included as a positive control. *C*, RT-PCR analysis of transcripts from *cnt2*, *cnt3*, and the *act1+* control. PP-*cnt2* and PP-*cnt3* are specific for the central domain of *cen2* and *cen3*, respectively.

methylation, a modification associated with active transcription, have been shown to be enriched in the small amount of histone H3 that remains within subkinetochore chromatin in wild-type cells (33).

DISCUSSION

Centromere-associated DNA has been shown to be transcribed in plants and vertebrates (7, 10). 30 years ago, EM studies demonstrated the presence of RNase-sensitive material at the base of kinetochore microtubules in newt lung cells (34). Since then, centromeric transcripts have been found to associate with kinetochore proteins (9, 10). In this study, we demonstrated an analogy between the central domain-associated CENP-A[Cnp1] chromatin of centromeres and genes whose promoters are associated with Hrp1[Chd1] in fission yeast. The fact that Hrp1[Chd1] promotes eviction of histone H3 at a set of promoters suggests that similar remodeling processes may occur at RNAPII promoters within centromeres and may contribute to exchange of canonical H3-containing nucleosomes for CENP-A[Cnp1]-containing nucleosomes (summarized in Fig. 5).

It is possible that transcription within centromeres occurs merely as a consequence of having RNAPII promoters whose presence or activation is critical to act as a seed for remodeling events that promote CENP-A[Cnp1] deposition. The resulting TUK transcripts are degraded by the exosome and thus may represent just nonfunctional by-products of tran-

scription. However, we cannot rule out the possibility that these unstable transcripts might also have a dedicated function in guiding some components of the CENP-A[Cnp1] deposition machineries and/or kinetochore complex to their cognate DNA sequences (10). Alternatively, the production of TUKs might influence chromatin modification within centromeric chromatin by processes analogous to those associated with cryptic or antisense noncoding RNA production in *S. cerevisiae* (35, 36). However, we found that the accumulation of TUKs in *pfs2-11*, *dhp1-1*, or *dis3-54* cells had no obvious effect on CENP-A[Cnp1] chromatin formation.[6] It is also conceivable that these transcripts are processed into a specific class of small RNAs that have a role in CENP-A[Cnp1] chromatin formation and/or kinetochore assembly analogous to how siRNAs derived from outer repeat transcripts induce heterochromatin formation. However, with our detection methods, we did not find evidence for the presence of small RNAs corresponding to the central domain of centromeres.[5]

Intriguingly, it has recently been reported that *S. cerevisiae* CENP-A[Cse4] also tends to associate with a number of RNAPII promoters where RNAPII binding is high (37); however, it is not known if these promoters share any structural or mechanistically related features with *S. cerevisiae* centro-

---

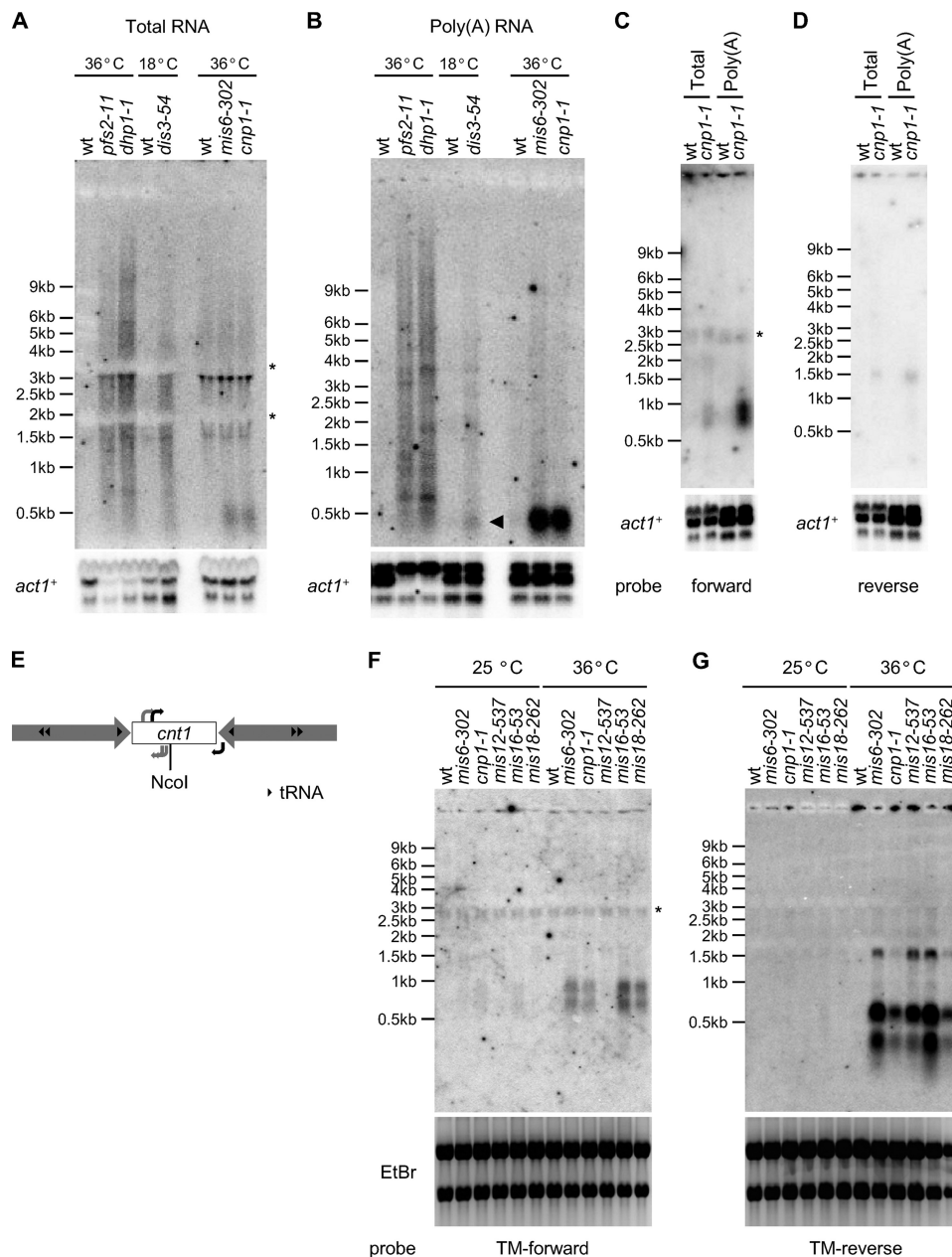[6] E. S. Choi and R. C. Allshire, unpublished data.

FIGURE 4. Northern and 5′-RACE/PCR analyses of central domain transcripts. *A*, Northern analysis of total RNAs in RNA-processing and kinetochore mutants. An RNA probe complementary to *cnt1* was used. Cells grown at the permissive temperature (25 °C for WT, *pfs2-11*, *dhp1-1*, *mis6-302*, and *cnp1-1* cells and 36 °C for WT and *dis3-54* cells) were shifted to the restrictive temperature (6 h at 36 °C for WT, *pfs2-11*, *dhp1-1*, *mis6-302*, and *cnp1-1* cells and 9 h at 18 °C for WT and *dis3-54* cells) before RNA extraction. *act1*⁺ was used as a loading control. *, rRNA interference with hybridization. For additional EtBr images, see supplemental Fig. S5A. *B*, the same *cnt1* probe used on poly(A) RNA. For additional EtBr images, see supplemental Fig. S5B. *C* and *D*, Northern analysis of total or poly(A) RNAs from WT and *cnp1-1* cells with an RNA probe complementary to the reverse strand (forward probe; *C*) or the forward strand (reverse probe; *D*) of the cc1/tm1 sequence, which is shared by *cnt1* and *cnt3*. *, nonspecific band. For additional EtBr images, see supplemental Fig. S5 (*C* and *D*). *E*, schematic representation of transcription start sites determined by 5′-RACE/PCR in WT and *cnp1-1* cells. *Black arrows*, transcription start sites identified in WT and *cnp1-1* cells; *gray arrows*, transcription start sites identified only in *cnp1-1* cells. *F* and *G*, Northern blots showing transcripts complementary to the TM-forward (*F*) or TM-reverse (*G*) probes in WT, *mis6-302*, *cnp1-1*, *mis12-537*, *mis16-53*, and *mis18-262* cells grown at permissive (25 °C) and restrictive (36 °C) temperatures. *, nonspecific band. EtBr staining confirmed equal loading.

meres that contribute to CENP-A^{Cse4} deposition. Interestingly, the *S. cerevisiae* centromeric protein Cbf1, which binds centromere DNA element I, functions as a transcription factor at the *MET16* promoter (38). In this regard, it is possible that RNAPII transcription might also occur within or close to centromeres of *S. cerevisiae*. In fact, cryptic transcription was detected close to *CEN3* in *S. cerevisiae* exosome mutants (39); it is not known if this is a general feature

of all *S. cerevisiae* centromeres. Recently, the human chromatin-remodeling factor FACT (facilitates chromatin transcription), whose function is implicated in transcription, was found to associate with affinity-purified CENP-A chromatin (40, 41) Moreover, depletion of FACT was found to impair incorporation of newly synthesized CENP-A in chicken cells (42). CHD1 was also found at centromeres in chicken cells and is required for centromeric localization of CENP-A in
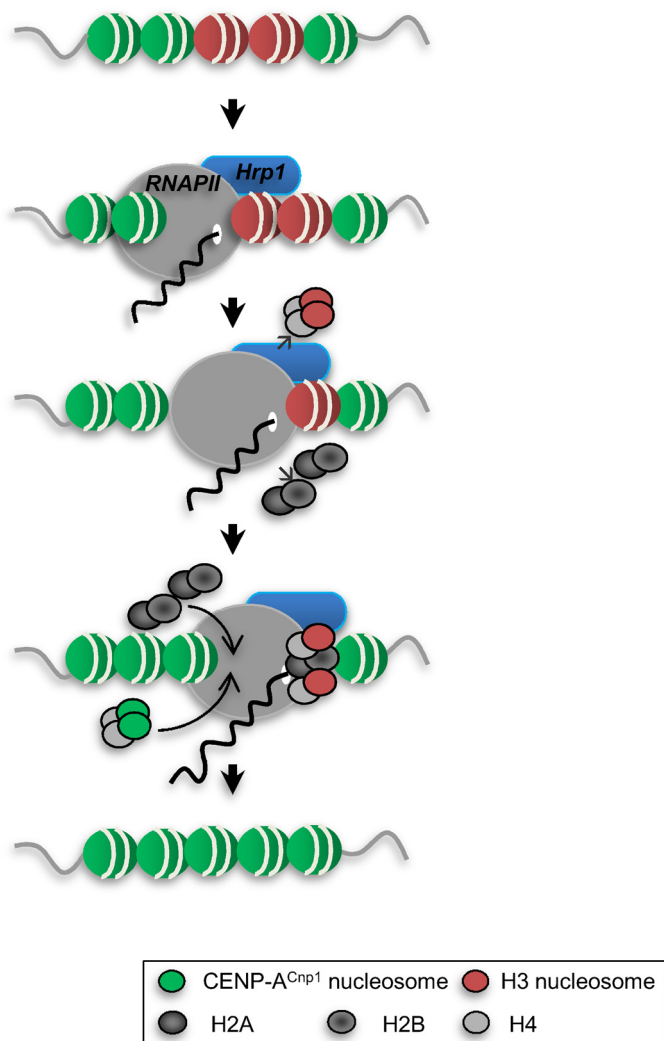
FIGURE 5. **Proposed model for the role of Hrp1 in the exchange of histone H3 with CENP-A^Cnp1 during RNAPII transcription.** Transcription from the promoters within centromeric chromatin acts to promote the replacement of histone H3 with CENP-A^Cnp1 through the associated remodeling factor Hrp1^Chd1. Similarly, Hrp1^Chd1 acts at some euchromatic promoters to replace H3 with CENP-A^Cnp1. The resulting centromeric transcripts are degraded by the exosome.

human cells (42). These observations, together with the analyses presented here, implicate RNAPII transcription and the associated remodeling activities in the replacement of histone H3 with CENP-A.

REFERENCES

1. Allshire, R. C., and Karpen, G. H. (2008) *Nat. Rev. Genet.* **9,** 923–937
2. Cleveland, D. W., Mao, Y., and Sullivan, K. F. (2003) *Cell* **112,** 407–421
3. Schueler, M. G., and Sullivan, B. A. (2006) *Annu. Rev. Genomics Hum. Genet.* **7,** 301–313
4. Folco, H. D., Pidoux, A. L., Urano, T., and Allshire, R. C. (2008) *Science* **319,** 94–97
5. Fujita, Y., Hayashi, T., Kiyomitsu, T., Toyoda, Y., Kokubu, A., Obuse, C., and Yanagida, M. (2007) *Dev. Cell* **12,** 17–30
6. Hayashi, T., Fujita, Y., Iwasaki, O., Adachi, Y., Takahashi, K., and Yanagida, M. (2004) *Cell* **118,** 715–729
7. Carone, D. M., Longo, M. S., Ferreri, G. C., Hall, L., Harris, M., Shook, N., Bulazel, K. V., Carone, B. R., Obergfell, C., O'Neill, M. J., and O'Neill, R. J. (2009) *Chromosoma* **118,** 113–125
8. Chueh, A. C., Northrop, E. L., Brettingham-Moore, K. H., Choo, K. H., and Wong, L. H. (2009) *PLoS Genet.* **5,** e1000354
9. Topp, C. N., Zhong, C. X., and Dawe, R. K. (2004) *Proc. Natl. Acad. Sci. U.S.A.* **101,** 15986–15991
10. Wong, L. H., Brettingham-Moore, K. H., Chan, L., Quach, J. M., Anderson, M. A., Northrop, E. L., Hannan, R., Saffery, R., Shaw, M. L., Williams, E., and Choo, K. H. (2007) *Genome Res.* **17,** 1146–1160
11. Chen, E. S., Saitoh, S., Yanagida, M., and Takahashi, K. (2003) *Mol. Cell* **11,** 175–187
12. Walfridsson, J., Bjerling, P., Thalen, M., Yoo, E. J., Park, S. D., and Ekwall, K. (2005) *Nucleic Acids Res.* **33,** 2868–2879
13. Simic, R., Lindstrom, D. L., Tran, H. G., Roinick, K. L., Costa, P. J., Johnson, A. D., Hartzog, G. A., and Arndt, K. M. (2003) *EMBO J.* **22,** 1846–1856
14. Konev, A. Y., Tribus, M., Park, S. Y., Podhraski, V., Lim, C. Y., Emelyanov, A. V., Vershilova, E., Pirrotta, V., Kadonaga, J. T., Lusser, A., and Fyodorov, D. V. (2007) *Science* **317,** 1087–1090
15. Durand-Dubief, M., and Ekwall, K. (2009) *Methods Mol. Biol.* **529,** 279–295
16. Walfridsson, J., Khorosjutina, O., Matikainen, P., Gustafsson, C. M., and Ekwall, K. (2007) *EMBO J.* **26,** 2868–2879
17. Pidoux, A. L., Richardson, W., and Allshire, R. C. (2003) *J. Cell Biol.* **161,** 295–307
18. Castillo, A. G., Mellone, B. G., Partridge, J. F., Richardson, W., Hamilton, G. L., Allshire, R. C., and Pidoux, A. L. (2007) *PLoS Genet.* **3,** e121
19. Pidoux, A. L., Choi, E. S., Abbott, J. K., Liu, X., Kagansky, A., Castillo, A. G., Hamilton, G. L., Richardson, W., Rappsilber, J., He, X., and Allshire, R. C. (2009) *Mol. Cell* **33,** 299–311
20. Williams, J. S., Hayashi, T., Yanagida, M., and Russell, P. (2009) *Mol. Cell* **33,** 287–298
21. Henikoff, S. (2008) *Nat. Rev. Genet.* **9,** 15–26
22. Dion, M. F., Kaplan, T., Kim, M., Buratowski, S., Friedman, N., and Rando, O. J. (2007) *Science* **315,** 1405–1408
23. Cam, H. P., Chen, E. S., and Grewal, S. I. (2009) *Cell* **136,** 610–614
24. Kloc, A., and Martienssen, R. (2008) *Trends Genet.* **24,** 511–517
25. Houseley, J., and Tollervey, D. (2009) *Cell* **136,** 763–776
26. Bühler, M., Haas, W., Gygi, S. P., and Moazed, D. (2007) *Cell* **129,** 707–721
27. Murakami, H., Goto, D. B., Toda, T., Chen, E. S., Grewal, S. I., Martienssen, R. A., and Yanagida, M. (2007) *PLoS One* **2,** e317
28. Wang, S. W., Stevenson, A. L., Kearsey, S. E., Watt, S., and Bähler, J. (2008) *Mol. Cell. Biol.* **28,** 656–665
29. Wang, S. W., Asakawa, K., Win, T. Z., Toda, T., and Norbury, C. J. (2005) *Mol. Cell. Biol.* **25,** 2288–2296
30. Shobuike, T., Tatebayashi, K., Tani, T., Sugano, S., and Ikeda, H. (2001) *Nucleic Acids Res.* **29,** 1326–1333
31. Saitoh, S., Takahashi, K., and Yanagida, M. (1997) *Cell* **90,** 131–143
32. Takahashi, K., Chen, E. S., and Yanagida, M. (2000) *Science* **288,** 2215–2219
33. Cam, H. P., Sugiyama, T., Chen, E. S., Chen, X., FitzGerald, P. C., and Grewal, S. I. (2005) *Nat. Genet.* **37,** 809–819
34. Rieder, C. L. (1979) *J. Cell Biol.* **80,** 1–9
35. Camblong, J., Iglesias, N., Fickentscher, C., Dieppois, G., and Stutz, F. (2007) *Cell* **131,** 1534–1545
36. Houseley, J., Rubbi, L., Grunstein, M., Tollervey, D., and Vogelauer, M. (2008) *Mol. Cell* **32,** 685–695
37. Lefrançois, P., Euskirchen, G. M., Auerbach, R. K., Rozowsky, J., Gibson, T., Yellman, C. M., Gerstein, M., and Snyder, M. (2009) *BMC Genomics*

10, 37

38. Hemmerich, P., Stoyan, T., Wieland, G., Koch, M., Lechner, J., and Diekmann, S. (2000) *Proc. Natl. Acad. Sci. U.S.A.* **97,** 12583–12588

39. Houseley, J., Kotovic, K., El Hage, A., and Tollervey, D. (2007) *EMBO J.* **26,** 4996–5006

40. Foltz, D. R., Jansen, L. E., Black, B. E., Bailey, A. O., Yates, J. R., 3rd, and

Cleveland, D. W. (2006) *Nat. Cell Biol.* **8,** 458–469

41. Izuta, H., Ikeno, M., Suzuki, N., Tomonaga, T., Nozaki, N., Obuse, C., Kisu, Y., Goshima, N., Nomura, F., Nomura, N., and Yoda, K. (2006) *Genes Cells* **11,** 673–684

42. Okada, M., Okawa, K., Isobe, T., and Fukagawa, T. (2009) *Mol. Biol. Cell* **20,** 3986–3995