LETTERS

RNA-interference-directed chromatin modification coupled to RNA polymerase II transcription

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RNA interference (RNAi) acts on long double-stranded RNAs (dsRNAs) in a variety of eukaryotes to generate small interfering RNAs that target homologous messenger RNA, resulting in their destruction. This process is widely used to 'knock-down' the expression of genes of interest to explore phenotypes[1–3]. In plants[3–5], fission yeast[6–8], ciliates[9,10], flies[11] and mammalian cells[12,13], short interfering RNAs (siRNAs) also induce DNA or chromatin modifications at the homologous genomic locus, which can result in transcriptional silencing or sequence elimination[14]. siRNAs may direct DNA or chromatin modification by siRNA–DNA interactions at the homologous locus[4,5]. Alternatively, they may act by interactions between siRNA and nascent transcript[15,16]. Here we show that in fission yeast (*Schizosaccharomyces pombe*), chromatin modifications are only directed by RNAi if the homologous DNA sequences are transcribed. Furthermore, transcription by exogenous T7 polymerase is not sufficient. Ago1, a component of the RNAi effector RISC/RITS complex, associates with target transcripts and RNA polymerase II. Truncation of the regulatory carboxy-terminal domain (CTD) of RNA pol II disrupts transcriptional silencing, indicating that, like other RNA processing events[17–19], RNAi-directed chromatin modification is coupled to transcription.

In plant and mammalian cells siRNAs homologous to the open reading frame of a gene results in post-transcriptional silencing, degrading transcripts by means of RNAi. However, siRNAs homologous to a gene's promoter can induce transcriptional silencing, resulting in the modification of DNA and/or chromatin[3–5]. siRNAs may hybridize to DNA and thereby recruit DNA/chromatin modifying activities that effect silencing[4,5,14]. Alternatively, the RNAi machinery may target nascent transcripts and cause chromatin modification on templates homologous to loaded siRNAs[15,16]. At fission yeast centromeres and the silent mating type locus, non-coding RNAs are generated by the transcription of both strands of related repeats[6,20]. These form dsRNAs, which are cleaved by Dicer (Dcr1) into siRNAs and then loaded into the Ago1 (Argonaute)-containing RITS (for RNA-induced initiation of transcriptional silencing) complex, which mediates RNAi[20,21]. Nascent transcripts may direct the RNAi machinery to the homologous locus, induce dimethylation of the surrounding chromatin on lysine 9 of histone H3 (H3K9me2) through Clr4, recruiting Swi6 (HP1) and thereby silencing transcription[15,16]. Components of RITS and RNA-dependent RNA polymerase (Rdp1) are known to associate with, and act in *cis* on, this silent chromatin[6,21,22]. However, Chp1 (a RITS component) and Swi6 bind H3K9me2 (ref. 23), and Rdp1 (and the RDRC (RNA-directed RNA polymerase complex)) interacts with RITS and requires Swi6 for chromatin association[24,25]. Because of this and the inherent self-enforcing nature of the process, it is difficult to determine whether nascent transcripts are required to mediate RNAi-directed chromatin modifications, and what additional interactions are involved.

Expression of a synthetic hairpin RNA homologous to a 280-base-pair (bp) region located within the *ura4*$^+$ gene (*sh-ura4-280*) induces Dicer-dependent transcriptional silencing of *ura4*$^+$ along with H3K9me2 of *ura4* chromatin and recruitment of Swi6 (ref. 7). To determine whether this hairpin-induced chromatin modification requires a homologous transcript, a strain containing a modified *ura4*$^+$ gene with an efficient transcription terminator module immediately upstream of the 280-bp *ura4* target region was used[26]. In this strain (Ter$^+$ *ura4*) the transcriptional terminator is inserted within an artificial intron, so that more than 99% of transcripts are terminated before the 280-bp region homologous to the *sh-ura4-280* trigger. A second strain (Ter-M5 *ura4*) contains a *cis*-acting mutation within the terminator, allowing 75% of transcripts to traverse the downstream 280-bp region of the gene[26] (Fig. 1a). Both strains also contain *ura4-DS/E* at the *ura4*$^+$ locus, which is fully transcribed but lacks the 280-bp region homologous to the *sh-ura4-280* trigger, thus providing a convenient internal control[7].

The construct expressing *sh-ura4-280* was introduced into both strains, and transcription of *ura4*$^+$, H3K9me2 modification of *ura4* chromatin and recruitment of Swi6 were assessed in wild-type strains in the presence and absence of the *sh-ura4-280* hairpin. In cells containing the Ter$^+$ *ura4* gene, truncated (*ura-T*), but not full-length (*ura4-FL*), transcript is detected in the presence or absence of *sh-ura4-280* (Fig. 1b). In Ter-M5 cells, full-length *ura4* transcript is lost in the presence of *sh-ura4-280* but expression of *ura4-DS/E* remains unaffected (Fig. 1b). Thus, expression of a hairpin target homologous to downstream DNA sequences does not affect Ter$^+$ *ura4*, whereas Ter-M5 *ura4* transcripts are repressed by *sh-ura4-280* expression.

Chromatin immunoprecipitation (ChIP) was used to assess the levels of H3K9me2 modification on the Ter$^+$ and Ter-M5 *ura4* genes relative to *ura4-DS/E*. H3K9me2 was detected only on Ter-M5 *ura4*, and only in strains expressing *sh-ura4-280* (Fig. 1c). *ura4* siRNAs were detected only in strains containing the *sh-ura4-280* construct (Fig. 1d). Thus, RNAi can induce chromatin modifications at a homologous locus only if transcripts traverse a region identical in sequence to the hairpin trigger and the resultant siRNAs. It is possible that the passage of RNA polymerase II (pol II) during transcription itself, by opening chromatin, provides access for siRNAs to underlying DNA sequences, thus allowing siRNA–DNA interactions[4,5,14]. Alternatively, Ago1-bearing siRNAs may bind homologous nascent transcripts and in so doing recruit chromatin-modifying activities through the Ago1-containing RITS and/or Rdp1-containing RDRC complex[15,16]. If opening the two DNA strands is sufficient, then an

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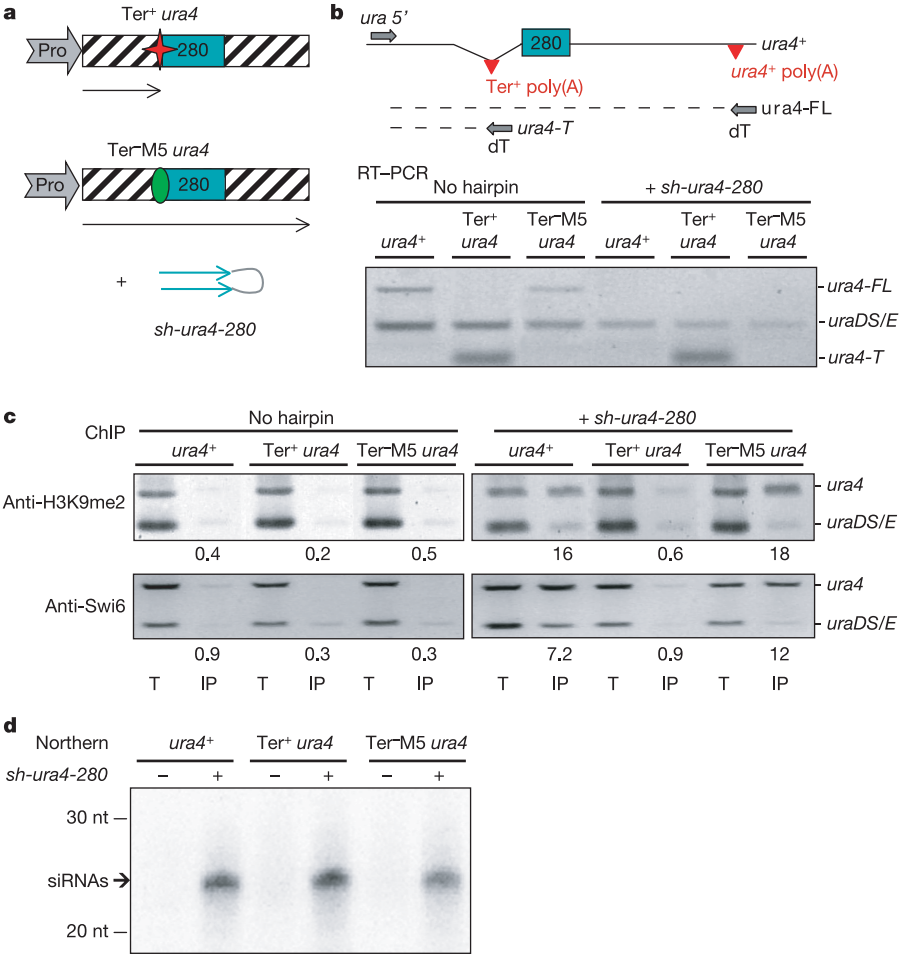

Figure 1 | Transcription of siRNA target is required to effect silent chromatin assembly. **a**, Strains containing the Ter^{+}-*ura4* gene with an efficient Ter^{+} or defective Ter-M5 terminator upstream of the 280-bp ura4 target region. **b**, RT–PCR analysis of *ura4*$^{+}$ transcripts on oligo(dT)-primed cDNA from RNA samples of the indicated strains; expression of *ura4*$^{+}$-terminated (*ura-T*) and full-length (*ura4-FL*) transcripts relative to the *ura4-DS/E* minigene in the same strains with or without *sh-ura4*-280 hairpin. **c**, ChIP analyses with anti-H3K9me2 and Swi6 antibodies over the *ura4* gene in the indicated strains. T, total extract; IP, immunoprecipitate. **d**, Detection of *sh-ura4-280*-generated siRNAs by northern blotting. nt, nucleotides.

exogenous RNA polymerase might allow siRNAs access to homologous chromatin. To test this, the *ura4* transcription unit was placed downstream of the bacteriophage T7 promoter (Fig. 2). T7 polymerase was constitutively expressed from the *adh1* promoter in the presence or absence of *sh-ura4-280* (Fig. 2a, and Supplementary Fig. S1a). *T7:ura4* transcripts are detected only in cells expressing T7 polymerase. The expression of *sh-ura4-280* did not reduce the level of *T7:ura4* transcripts significantly (Fig. 2b), although RNAi is active because *ura4* siRNAs are readily detected (Fig. 2d). In addition, no H3K9me2 or Swi6 could be detected on *T7:ura4* chromatin (Fig. 2c, and Supplementary Fig. S1b) in cells expressing *sh-ura4-280* homologous siRNAs (Fig. 2d), although histone H3 is present on the *T7:ura4* gene (Supplementary Fig. S1c). Lack of RNAi-directed chromatin modification of the *T7:ura4* template may reflect the absence of features normally associated with endogenous RNA pol II transcription. T7 and RNA pol II transcription and the resulting transcripts differ in many respects; regardless of this, transcription of target chromatin alone is not sufficient to mediate RNAi-directed chromatin modifications on homologous chromatin. This indicates that transcripts generated by, or associated with, a specific RNA polymerase might be required.

RNA pol II is responsible for the generation of fission yeast centromere repeat transcripts that are processed by RNAi into homologous siRNAs. A mutation (*rpb7-1*) in Rpb7, a small pol II subunit, leads to a loss of these transcripts and siRNAs (K.E., unpublished observations). The CTD of the large subunit of pol II (Rpb1) contains multiple conserved YSPTSPS heptad repeats, the phosphorylation state of which regulates the binding of various mRNA processing factors, thus coupling mRNA processing to transcription[17–19]. In *Saccharomyces cerevisiae* the deletion of up to

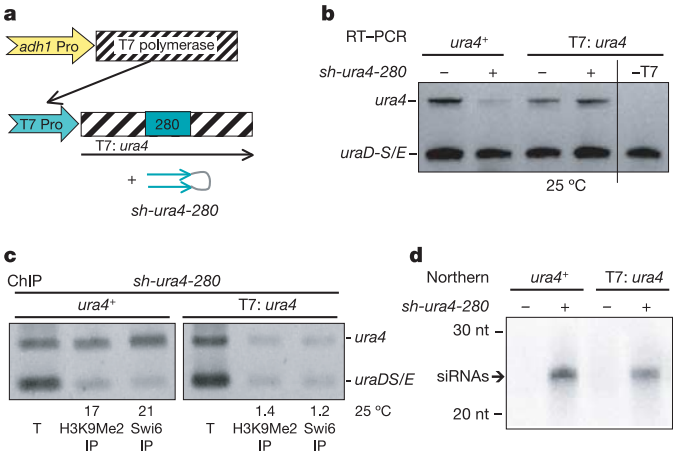

Figure 2 | Transcription by T7 polymerase is not sufficient for RNAi-directed chromatin modification. **a**, The *ura4*$^{+}$ promoter was replaced with the bacteriophage T7 promoter (T7:*ura4*). T7 polymerase was constitutively expressed from the *adh1* promoter in the presence or absence of *sh-ura4-280*. Cells were grown at 25 °C. **b**, RT–PCR analysis of *ura4*$^{+}$ transcripts was performed on oligo(dT)-primed cDNA from RNA samples from the indicated strains expressing (+) or not (−) *sh-ura4280*-generated siRNAs. **c**, ChIP analyses with anti-H3K9me2 and anti-Swi6 antibodies over the *ura4* gene in the indicated strains. T, total extract; IP, immunoprecipitate. **d**, Detection of *sh-ura4-280* generated siRNAs by northern blotting.

16 of the 26 CTD heptad repeats from pol II results in compromised RNA polymerase functions[27]. If pol II has a specific function in mediating RNAi-mediated chromatin modification, then cells bearing a defective pol II might display aberrant silencing of marker genes at centromeres. To examine this, a strain was constructed with 17 of the 28 CTD heptad repeats deleted and simultaneously epitope-tagged (*rpb1-11*, see Methods; Fig. 3a). This strain was slow-growing but viable at all temperatures tested and was clearly defective in its ability to silence centromeric *ura4*$^+$ and *ade6*$^+$ markers as revealed by increased growth on plates lacking uracil (−ura) and the appearance of white ade$^+$ colonies, respectively (Fig. 3b, left). Consistent with this was the detection of increased levels of *cen1:ura4*$^+$ transcripts (Fig. 3b, right) and decreased levels of H3K9me2 associated with centromere repeats (Fig. 3c). However, centromeric transcripts do not accumulate appreciably in *rpb1-11* compared with *dcr1Δ*, and centromeric siRNAs are readily detected as in the wild type (Fig. 3d). This indicates that although RNAi remains active it is unable to induce chromatin modifications efficiently on homologous sequences. The phenotype of *rpb1-11* is clearly distinct from that of *rpb7-1*, which is defective in centromeric transcription and siRNA production, causing a failure of transcriptional silencing (K.E., unpublished observations). Microarray expression profiling indicated that none of the known genes involved in RNAi-directed chromatin silencing are significantly affected in *rpb1-11* cells in comparison with the wild type, and few genes were affected to any great extent (Supplementary Table 1). Thus, the CTD truncation does not seem to cause a substantial general defect in transcription. This indicates that the CTD of pol II might act downstream of RNAi to stabilize interactions between RNAi components, the nascent transcript and possibly the pol II holoenzyme to induce chromatin modifications. RNAi components might require intact pol II to fully engage a chromatin-associated nascent transcript, or intact pol II

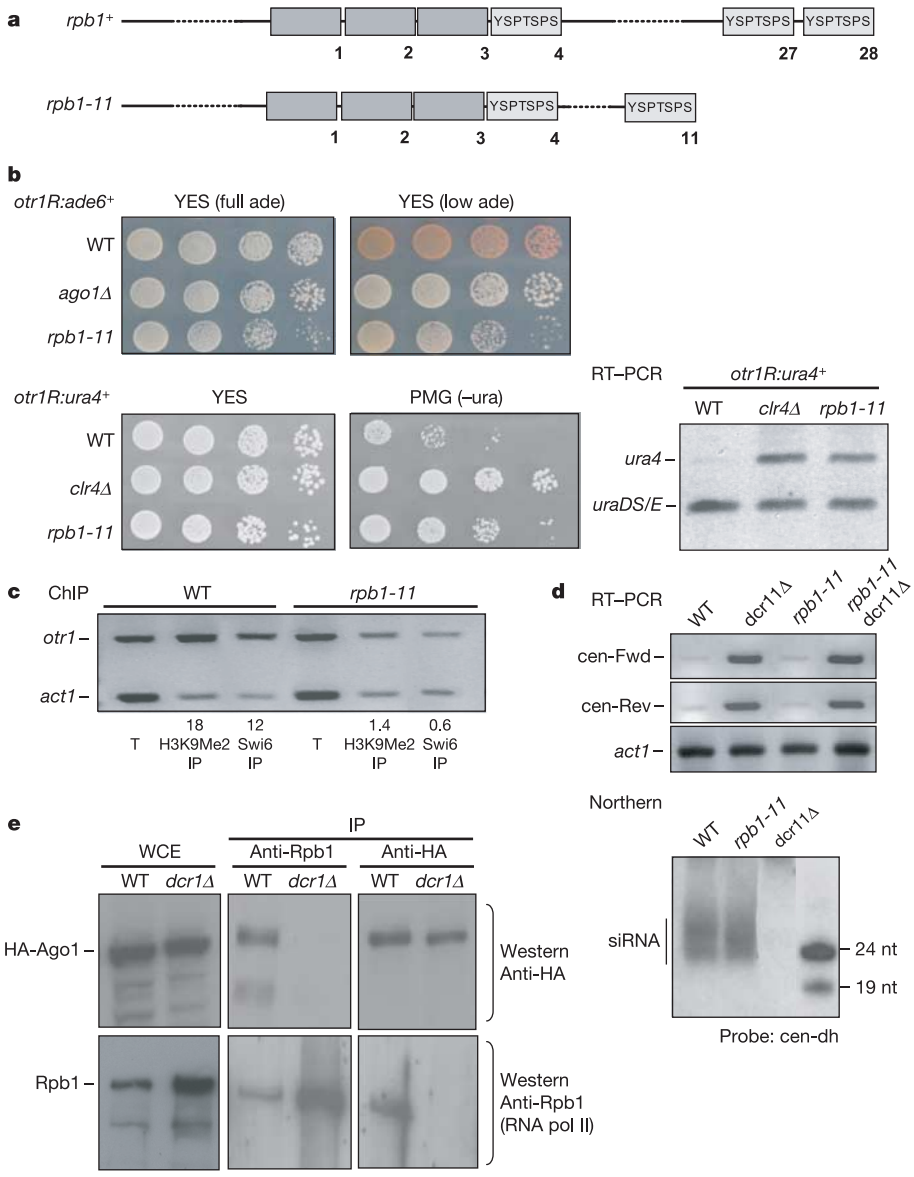

Figure 3 | RNA pol II CTD truncation affects centromeric silent chromatin. **a**, *rpb1-11* retains 11 of 28 heptad repeats. **b**, Left: growth assay of indicated strains with *ade6*$^+$ or *ura4*$^+$ genes in *cen1* outer repeat on non-selective (YES), limiting adenine (YES (low ade)) or without uracil (PMG (−ura)). Right: RT–PCR analysis of *cen1otr1:ura4*$^+$ transcripts performed on RNA from the indicated strains. WT, wild-type. **c**, ChIP analyses of H3K9me2 and Swi6 over the *cen1* outer repeat region (*otr1*) relative to the actin gene (*act1*). T, total extract; IP, immunoprecipitate. **d**, Detection of centromeric repeat transcripts and siRNAs by RT–PCR (top) and northern blotting (bottom), respectively. **e**, Immunoprecipitation and detection of HA-Ago1 and Rpb1 by western blot analysis.

might be specifically required to synthesize a transcript in a form that can effectively associate with RNAi components. Argonaute (PAZ/PIWI domain) proteins enter RISC (or RITS) complexes and use loaded siRNAs to guide RISC/RITS to target RNAs. Immunoprecipitates of HA-Ago1 were found by western blot analyses to contain pol II (Rpb1); reciprocal to this, HA-Ago1 was detected in immunoprecipitates of pol II. This interaction also required siRNA-loaded RITS because Ago1 and pol II do not immunoprecipitate together from cells lacking Dicer (Fig. 3e).

To determine whether Ago1 associates with chromatin targeted for silencing by RNAi, ChIPs were performed with anti-Ago1 antibodies or HA-Ago1. Ago1 associated with centromeric outer repeats in wild-type, but not *dcr1Δ*, cells (Fig. 4a, top). Ago1 also showed *sh-ura4-280* siRNA and transcription-dependent association with the *ura4* gene (Fig. 4a, bottom). Ago1, but not Rad21, associated with centromeric *otr* transcripts, but not with control transcripts (*act1*), in wild-type cells, and not in cells lacking siRNAs (*dcr1Δ*) (Fig. 4b). The association of Ago1 with centromeric chromatin was sensitive to RNase (Fig. 4c). In addition, this association was reduced in strains carrying a truncated pol II CTD (*rpb1-11*) (Fig. 4d). Consistent with previous reports[20], immunolocalization shows that HA-Ago1 is concentrated at centromeres in the nucleus, as shown by localization with centromere-specific CENP-A^{Cnp1} (Fig. 4e, and Supplementary Fig. S2).

Taken together, these data show that, in fission yeast, RNAi requires the transcription of a homologous target to direct chromatin modifications. Opening DNA by T7 pol transcription does not allow modification of the target chromatin to occur. T7 pol might deal with impeding nucleosomes differently, or T7 pol transcripts might not be packaged or processed in the same manner as RNA pol II transcripts, rendering them immune to RNAi. The fact that truncation of the pol II CTD affects RNAi-directed chromatin modifications and association of Ago1 with centromeric repeats, without noticeably affecting centromere repeat transcription or siRNA generation, indicates that pol II transcription might facilitate the conversion of RNAi signals into chromatin modification. Many different factors associate with pol II through its CTD during distinct stages of transcription[17–19]; the pol II complex might provide a scaffold that promotes interactions between Ago1/RITS-borne siRNA and target pol II transcripts, leading to the efficient modification of occupied chromatin (see model in Supplementary Fig. S3). Indeed, it is known that RNA processing and export seem to be orchestrated with respect to ongoing transcription[17–19]. Our data indicate that RNAi-directed chromatin modification is another example of an RNA processing event that occurs co-transcriptionally, and offer an explanation for the apparent paradox that RNA pol II is not only required for transcriptional activity but is pivotal in transcriptional silencing. Moreover, plants have even evolved a distinct RNA polymerase (pol IV) required for RNAi-dependent chromatin silencing of certain repeat sequences[28,29].

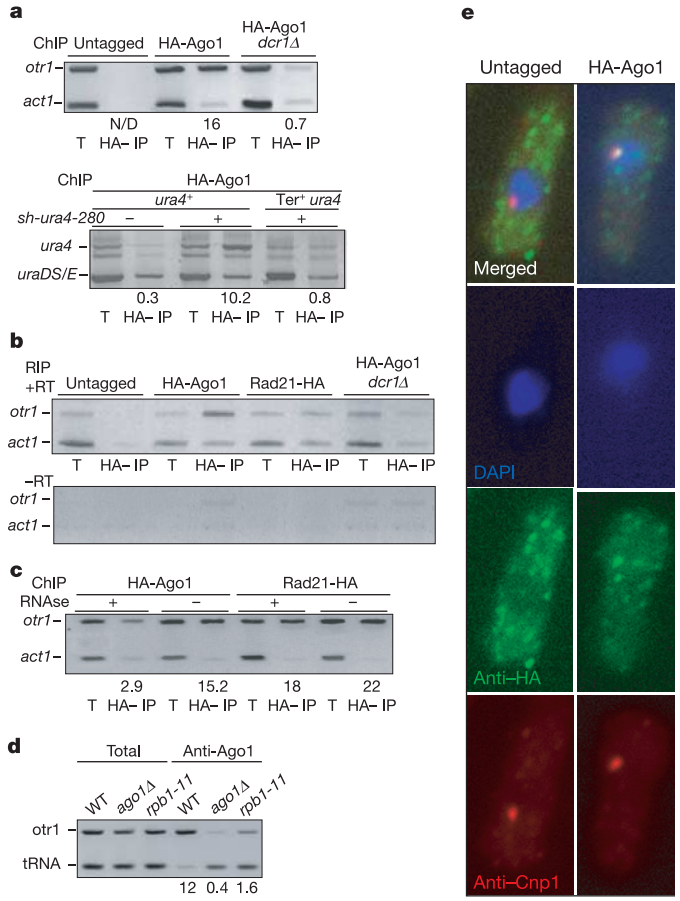

Figure 4 | Association of Ago1 with chromatin is RNase sensitive and dependent on transcription and pol II. **a**, ChIP detecting HA-Ago1 or Rad21-HA on *cen1* outer repeat (top) or *ura4*$^{+}$ with (+) or without (−) *sh-ura4-280* (bottom). **b**, RT–PCR of anti-HA immunoprecipitated RNA to detect HA-Ago1-associated and Rad21-HA-associated centromeric (*otr1*) versus actin (*act1*) transcripts. **c**, ChIP analyses of HA-Ago1 or Rad21-HA on *otr1* in the indicated strains treated (+) or not (−) with RNase. T, total extract; IP, immunoprecipitate. **d**, ChIP analyses of Ago1 on *otr1* relative to a transfer RNA gene in the indicated strains. WT, wild-type. **e**, Localization of HA-Ago1 and Cnp1 together at centromeres. DAPI, 4,6-diamidino-2-phenylindole.

METHODS

Standard techniques. Standard procedures were used for fission yeast growth, genetics and manipulations.

Yeast strains. *S. pombe* strains used are listed in Supplementary Table 2. Deletion of the 17 heptad repeats from Rpb1 and 3 × HA tagging of Ago1 was achieved by standard methods with the indicated primers (Supplementary Table 3). The KAN marker of the 3 × HA Ago1 tag was subsequently swapped to the nourseothricin (NAT) marker with indicated primers (Supplementary Table 3).

Reverse transcriptase polymerase chain reaction (RT–PCR). Total RNA was prepared from strains grown in YES medium at 25 or 32 °C and RT–PCR was performed as described previously[7]. The *ura4* and *ura4-DS/E* PCR products were separated on 1.5% agarose gels and poststained with ethidium bromide. Quantification of bands was performed with the Eastman Kodak EDAS 290 system and 1D Image Analysis software. Analysis was performed two to four times at each temperature, and average values from these experiments are presented.

Western blotting and immunoprecipitations. Antibodies for western blotting were diluted in PBS containing Tween as follows: anti-HA and anti-Rpb1 (monoclonal ARNA-3 from Research Diagnostics detecting residues 797–811 of Rpb1), diluted 1:300; anti-unmodified RNA pol II (8WG16), 1:1,000 (in 5% milk). Blots were developed with enhanced chemiluminescence reagents (Amersham Biosciences). Immunoprecipitations were performed as described previously[30].

Chromatin immunoprecipitation. ChIP was performed as described previously[7] except for the following modifications: cells were converted to spheroplasts by incubation for 25 min at 10^8 cells ml^{-1} in PEMS (100 mM PIPES pH 7, 1 mM EDTA, 1 mM $MgCl_2$, 1.2 M sorbitol) containing 0.4 mg ml^{-1} zymolyase-100T at 36 °C. Cells were washed twice in PEMS; cell pellets were frozen at −20 °C (ref. 30). Thereafter the standard ChIP procedure was followed. Antibodies against H3K9me2, Swi6 and HA were used as described previously[7]. Quantification of bands was performed with the Eastman Kodak EDAS 290 system and 1D Image Analysis software.

ChIP with an RNase step. ChIPs were performed with a monoclonal antibody against HA (12CA5) and using methods previously described, with the following exceptions. The crosslinking time was reduced from 15 min to 5 min when RNase treatment was performed. When an RNase treatment step was included, cross-linked chromatin from the same experiment was treated with either 7.5 U of RNase A and 300 U of RNase T1 (RNase A/T1 cocktail; Ambion) or an equivalent volume of RNase storage buffer (10 mM HEPES pH 7.2, 20 mM NaCl, 0.1% Triton X-100, 1 mM EDTA, 50% v/v glycerol). After incubation at 25 °C for 30 min, immunoprecipitations were performed as above.

RNA immunoprecipitation. RNA immunoprecipitation was performed as described[24].

Small RNA preparation and detection. Exponential-phase yeast cells were subjected to RNA extraction with TRIZOL (Invitrogen) and precipitation with propan-2-ol. Samples were resuspended in 50% formamide; 35 μg of total RNA was loaded on a 17.5% denaturating polyacrylamide gel containing 7 M urea. ^{32}P-labelled DNA probes complementary to centromeric *dh* repeats or the *ura4*$^+$ *Stu*I/*Eco*RV region were generated with a Random Primed DNA-labelling kit (Roche). The probes were hybridized to the membranes overnight at 42 °C in a rotating oven and washed twice with 2 × SSC, 2% SDS at 50 °C. Phosphor screens or films were exposed for a minimum of 3 h. DNA oligonucleotides of 24/20 nucleotides or 22 nucleotides in length complementary to the *dh* repeat or the *ura4*$^+$ *Stu*I/*Eco*RV region were used as markers.

Cytology. Immunofluorescence was performed as described[30], cells were fixed for 15–20 min in 3.7% freshly prepared formaldehyde for staining. The following antibodies were used: sheep anti-Cnp1 antiserum (dilution 1:300), mouse 12CA5 anti-HA (dilution 1:30). Secondary antibodies conjugated with fluorescein isothiocyanate (Sigma-Aldrich) or Alexa488 (Molecular Probes) were used at dilutions of 1:100 or 1:1,000. Microscopy was performed as described[30].

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1. Hannon, G. J. & Rossi, J. J. Unlocking the potential of the human genome with RNA interference. *Nature* **431,** 371–378 (2004).
2. Mello, C. C. & Conte, D. Jr. Revealing the world of RNA interference. *Nature* **431,** 338–342 (2004).
3. Baulcombe, D. RNA silencing in plants. *Nature* **431,** 356–363 (2004).
4. Jones, L., Ratcliff, F. & Baulcombe, D. C. RNA-directed transcriptional gene silencing in plants can be inherited independently of the RNA trigger and requires Met1 for maintenance. *Curr. Biol.* **11,** 747–757 (2001).
5. Mette, M. F., Aufsatz, W., van der Winden, J., Matzke, M. A. & Matzke, A. J. Transcriptional silencing and promoter methylation triggered by double-stranded RNA. *EMBO J.* **19,** 5194–5201 (2000).
6. Volpe, T. A. *et al.* Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. *Science* **297,** 1833–1837 (2002).
7. Schramke, V. & Allshire, R. Hairpin RNAs and retrotransposon LTRs effect RNAi and chromatin-based gene silencing. *Science* **301,** 1069–1074 (2003).
8. Lippman, Z. & Martienssen, R. The role of RNA interference in heterochromatic silencing. *Nature* **431,** 364–370 (2004).
9. Mochizuki, K., Fine, N. A., Fujisawa, T. & Gorovsky, M. A. Analysis of a piwi-related gene implicates small RNAs in genome rearrangement in *Tetrahymena*. *Cell* **110,** 689–699 (2002).
10. Mochizuki, K. & Gorovsky, M. A. Small RNAs in genome rearrangement in *Tetrahymena*. *Curr. Opin. Genet. Dev.* **14,** 181–187 (2004).
11. Pal-Bhadra, M. *et al.* Heterochromatic silencing and HP1 localization in *Drosophila* are dependent on the RNAi machinery. *Science* **303,** 669–672 (2004).
12. Kawasaki, H. & Taira, K. Induction of DNA methylation and gene silencing by short interfering RNAs in human cells. *Nature* **431,** 211–217 (2004).
13. Morris, K. V., Chan, S. W., Jacobsen, S. E. & Looney, D. J. Small interfering RNA-induced transcriptional gene silencing in human cells. *Science* **305,** 1289–1292 (2004).
14. Matzke, M. A. & Birchler, J. A. RNAi-mediated pathways in the nucleus. *Nature Rev. Genet.* **6,** 24–35 (2005).
15. Martienssen, R. A. Maintenance of heterochromatin by RNA interference of tandem repeats. *Nature Genet.* **35,** 213–214 (2003).
16. Grewal, S. I. & Moazed, D. Heterochromatin and epigenetic control of gene expression. *Science* **301,** 798–802 (2003).
17. Orphanides, G. & Reinberg, D. A unified theory of gene expression. *Cell* **108,** 439–451 (2002).
18. Proudfoot, N. New perspectives on connecting messenger RNA 3′ end formation to transcription. *Curr. Opin. Cell Biol.* **16,** 272–278 (2004).
19. Reed, R. Coupling transcription, splicing and mRNA export. *Curr. Opin. Cell Biol.* **15,** 326–331 (2003).
20. Noma, K. *et al.* RITS acts in cis to promote RNA interference-mediated transcriptional and post-transcriptional silencing. *Nature Genet.* **36,** 1174–1180 (2004).
21. Verdel, A. *et al.* RNAi-mediated targeting of heterochromatin by the RITS complex. *Science* **303,** 672–676 (2004).
22. Partridge, J. F., Borgstrom, B. & Allshire, R. C. Distinct protein interaction domains and protein spreading in a complex centromere. *Genes Dev.* **14,** 783–791 (2000).
23. Partridge, J. F., Scott, K. S., Bannister, A. J., Kouzarides, T. & Allshire, R. C. *cis*-acting DNA from fission yeast centromeres mediates histone H3 methylation and recruitment of silencing factors and cohesin to an ectopic site. *Curr. Biol.* **12,** 1652–1660 (2002).
24. Motamedi, M. R. *et al.* Two RNAi complexes, RITS and RDRC, physically interact and localize to noncoding centromeric RNAs. *Cell* **119,** 789–802 (2004).
25. Sugiyama, T., Cam, H., Verdel, A., Moazed, D. & Grewal, S. I. RNA-dependent RNA polymerase is an essential component of a self-enforcing loop coupling heterochromatin assembly to siRNA production. *Proc. Natl Acad. Sci. USA* **102,** 152–157 (2005).
26. Aranda, A. & Proudfoot, N. Transcriptional termination factors for RNA polymerase II in yeast. *Mol. Cell* **7,** 1003–1011 (2001).
27. Scafe, C., Nonet, M. & Young, R. A. RNA polymerase II mutants defective in transcription of a subset of genes. *Mol. Cell. Biol.* **10,** 1010–1016 (1990).
28. Herr, A. J., Jensen, M. B., Dalmay, T. & Baulcombe, D. C. RNA polymerase IV directs silencing of endogenous DNA. *Science* **308,** 118–120 (2005).
29. Onodera, Y. *et al.* Plant nuclear RNA polymerase IV mediates siRNA and DNA methylation-dependent heterochromatin formation. *Cell* **120,** 613–622 (2005).
30. Pidoux, A. L., Richardson, W. & Allshire, R. C. Sim4: a novel fission yeast kinetochore protein required for centromeric silencing and chromosome segregation. *J. Cell Biol.* **161,** 295–307 (2003).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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RETRACTION

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Formation of zirconium metallic glass

Jianzhong Zhang & Yusheng Zhao

Nature 430, 332–335 (2004)

In this Letter, we concluded, primarily on the basis of energy-dispersive X-ray diffraction, that metallic glass could be formed in pure zirconium under high pressure and temperature conditions. However, careful observations using an angular-dispersive method and imaging-plate detector, together with X-ray-transparent anvils (H. Saitoh, T. Hattori, H. Kaneko, Y. Okajima and W. Utsumi, unpublished results), have revealed that our conclusion was in error. We are now convinced that the disappearance of diffraction lines of zirconium observed in our energy-dispersive experiments should be interpreted instead as rapid crystal growth at temperatures above that of the ω–β phase transformation.

Our original misinterpretation was partly due to our assumption that significant crystalline growth could not occur at temperatures of less than one-third of the melting point of zirconium. To a greater extent, it was a result of the limitations of the experimental techniques then available, which only allowed viewing of a very narrow window of Debye diffraction rings and therefore increased the probability of missing the Bragg spots. The state-of-the-art techniques used by Saitoh *et al.* at the Synchrotron Radiation Research Center, Japan Atomic Energy Institute, Hyogo, are superior for high-pressure research on non-crystalline materials.

We thank our Japanese colleagues for their careful work and for inviting us to observe their high pressure and temperature synchrotron X-ray diffraction experiments at beamline BL14B1 of SPring-8.

RETRACTION

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Negative lattice expansion from the superconductivity–antiferromagnetism crossover in ruthenium copper oxides

A. C. Mclaughlin, F. Sher & J. P. Attfield

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This Letter described several notable physical properties for low-doped ruthenium copper oxides, from which conclusions concerning superconductivity in copper oxides were drawn. A key result was the observation of an unusual, negative, expansion of the lattice parameters and volume at temperatures below the Ru spin-ordering transition, T_{Ru}, as shown in Figure 3. Unfortunately, we have discovered that a discrepancy in the algorithm for fitting the diffraction data led to erroneous shifts in the cell parameters and resulted in the apparent negative expansion. We retract the claim of negative lattice expansion and the consequent inferences concerning the existence of two states in the pseudogap regime of the copper oxide electronic phase diagram. However, an anomalous expansion of the separation between copper oxide planes below T_{Ru}, as shown in Figure 3b, is reproducible and has subsequently been observed in other ruthenium copper oxide samples. Our results concerning large magnetoresistances and successive Ru and Cu spin-ordering transitions are not affected, so Figures 1 and 2 and the Supplementary Figures remain valid.

We thank S. Kimber and A. Williams for undertaking further calculations to determine the nature of the fitting problem.

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RNA-interference-directed chromatin modification coupled to RNA polymerase II transcription

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An earlier paper[1] by two of us, in which it was concluded that expression of a hairpin RNA homologous to *ura4* RNA in *Schizosaccharomyces pombe* results in the production of siRNAs that bring about methylation of histone H3 on lysine 9 and recruitment of Swi6 to the *ura4* gene, has been retracted[2]. This is because we are unable to reproduce the experiments involving expression of hairpin *ura4* RNA because the plasmid *sh-ura4*SE-280, or fission yeast strains that contain it, do not exist. Attempts to reproduce these observations with other *ura4*-hairpin constructs have failed.

As a result, we cannot verify the observations reported in our Letter to *Nature* and shown in Figures 1, 2 and 4a (lower panel) because these utilize the same plasmid *sh-ura4*-280 hairpin construct. We therefore retract the conclusions that transcription of an siRNA homologous target is required to bring about silent chromatin assembly, that transcription of an siRNA homologous target by T7 is not sufficient to bring about silent chromatin assembly, and that transcription of an siRNA homologous target is required to allow association of Ago1 with that target.

However, we have confirmed that truncation of the RNApol II CTD (removal of 17/28 heptad repeats) does alleviate silencing at centromeres, that centromeric siRNAs remain detectable (Figure 3), and that RNA pol II and Ago1 co-immunoprecipitate. In addition, the expression-profiling data generated for *rpb1-11* are robust.

V.S. has declined to sign this retraction as she maintains that the original conclusions are correct.

1. Schramke, V. & Allshire, R. C. Hairpin RNAs and retrotransposon LTRs effect RNAi and chromatin-based gene silencing. *Science* 301, 1069–1074 (2003).
2. Schramke, V. & Allshire, R. C. Retraction. *Science* 310, 49 (2005).