Silencing in *trans*: position matters in fission yeast

Monika Gullerova & Nick J. Proudfoot

RNA interference (RNAi)-mediated gene silencing is a widespread mechanism in eukaryotes that operates at several levels of gene expression (reviewed in Carthew & Southeimer, 2009). In metazoans, small interfering RNAs (siRNAs) derived from longer double-stranded RNAs (dsRNAs) target mRNA for degradation in the cytoplasm through the activation of the RISC complex. In this case, the dsRNA is normally exogenous, possibly of viral origin or introduced experimentally by transfection. Alternatively, nuclear dsRNA synthesis—often arising from hairpin structures in the introns of mRNA encoding genes—can give rise to cytoplasmic microRNAs and similarly activate RISC to either degrade mRNA or block its translation. By contrast, in lower eukaryotes RNAi seems to operate predominantly at the gene level, as nuclear dsRNA directly targets chromatin repression. Thus, in fission yeast RNAi is initiated by the production of siRNAs from dsRNA by the ribonuclease III enzyme Dicer (Dcr1). These siRNAs are loaded onto the Argonaute-containing RNAi transcriptional silencing complex (RITS), which somehow targets nascent transcripts (Buhler *et al*, 2006) and also recruits RNA-dependent RNA polymerase (Rdp1)-containing complex RDRC (Motamedi *et al*, 2004). Rdp1 effectively amplifies the Dcr1 dsRNA substrate. The RNAi machinery subsequently recruits the histone methyltransferase (Clr4)-containing complex CLRC to target loci, thereby promoting H3 Lys9 methylation (Hong *et al*, 2005). In turn, this forms a platform for heterochromatin binding proteins Swi6 (HP1 homologue), Chp1 and Chp2, creating a nucleation site from where heterochromatin can spread to adjacent regions.

Interestingly, the distinction between metazoans, which seem to predominantly use a post-transcriptional RNAi mechanism, and fission yeast, which uses a more direct transcriptional RNAi mechanism, could be less clear-cut than previously thought. Recent results in mammals suggest a role of RNAi in transcriptional gene silencing through the targeting of promoter regions (Han *et al*, 2007) and—in the February

issue of *EMBO reports*—the Allshire group showed that *Schizosaccharomyces pombe* can repress gene expression through a *trans* effect that is reminiscent of mammalian post-transcriptional gene silencing (Fig 1; Simmer *et al*, 2010). Both transcriptional gene silencing and post-transcriptional gene silencing are also well known to coexist in plants (Baulcombe, 2004).

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Previous studies in fission yeast suggested that RNAi gene silencing is only efficient when the dsRNA that induces the RNAi response comes from the same site as the silenced genes—that is, in *cis*. When dsRNA was made at a separate location (that is, in *trans*)—either elsewhere in the genome or from a transformed plasmid—RNAi effects were only detectable in combination with other genetic modifications, such as deletion of *eri1* (Buhler *et al*, 2006) or overexpression of *swi6* (Iida *et al*, 2008). However, the

new study by Simmer and colleagues brings further insight into the mechanism of RNAi-mediated gene silencing in *trans*. In particular, the authors show that exogenous hairpin RNA made in *trans* induces heterochromatin formation in otherwise unmodified wild-type cells. They then detect secondary siRNAs that are not derived from the hairpin sequence but are still close to the target gene. This class of siRNA is dependent on Rdp1 activity *in vivo*. Furthermore, they describe the presence of Ago1-associated siRNAs that do not necessarily lead to gene silencing (Simmer *et al*, 2010).

The ectopic expression of a long GFP hairpin RNA had been shown previously to lead to dsRNA production and post-transcriptional silencing of *adh1:gfp* mRNA. This silencing had no effect on the transcriptional rate of the target GFP gene, did not lead to heterochromatin formation and occurred in a RITS-independent manner (Sigova *et al*, 2004). By contrast, tethering of Tas3 (RITS complex) to a target *ura4* gene (that is, in *cis*) was shown to induce the production of siRNA, the recruitment of RNAi components and the *cis*-silencing of the *ura4* target gene. However, a second separate (that is, in *trans*) *ura4* allele was not silenced, suggesting the negative control of *trans*-silencing. In fact, only the deletion of *eri1* allowed partial silencing of the second *ura4* copy, suggesting that *trans*-silencing is influenced by additional factors (Buhler *et al*, 2006). Another study showed that siRNA produced from long hairpin RNA can induce *trans*-silencing, but it depends on local chromatin structure and correlates with antisense transcription at the

Fig 1 | *Trans*-silencing depends on the position of the target gene. siRNAs generated from a hairpin (shown in red) have the ability to induce silencing through the RNAi pathway; the siRNAs generated are shown below the centromere. *Trans*-silencing is only efficient if the target gene (shown as a blue arrow) is located near heterochromatic loci, such as centromeres, or positions of convergent transcription on chromosomal arms. CLRC, Clr4-containing complex; RDRC, RNA-dependent RNA polymerase-containing complex; RITS, RNAi transcriptional silencing complex.

target locus. Furthermore, *trans*-silencing is dependent on dsRNA synthesis by RDRC, although the production of primary siRNA from a hairpin is independent of Rdp1. This hairpin-induced siRNA *trans*-silencing was also enhanced by transient overexpression of Swi6 (Iida *et al.*, 2008).

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The Allshire group has now shown that ectopic expression of *ura4*⁺ hairpin RNA (U-HP) can induce heterochromatin formation in *trans* in the absence of any other genetic modifications. The expression of U-HP produces *ura4* siRNA but does not silence *ura4* at its endogenous position. However, it does lead to significant silencing of *ura4* when it is integrated close to a pericentromeric repeat, which is consistent with previous observations (Iida *et al.*, 2008). The expression of a GFP hairpin (GFP-HP) in the *arg3::ura4⁺GFP* strain also produces specific *gfp* siRNA, which are loaded onto Ago1 in the RITS complex. Interestingly, both *gfp* and *ura4* siRNAs can be detected, suggesting that secondary siRNA can also be produced in fission yeast *in vivo* (Fig 1). Sequence analysis revealed that this secondary siRNA pool is dependent on Rdp1 activity, which is consistent with previous data (Iida *et al.*, 2008). Simmer and co-workers also show that hairpin *gfp* siRNA partly represses the expression of *arg3::ura4⁺GFP* grown on 5-FOA plates, but does not significantly decrease the steady state RNA levels of *ura4* in cells grown in non-selective medium. Similarly, hairpin *gfp* siRNA can silence the *ade6* gene in an *ade6-GFP* strain.

Colonies grown in low adenine give rise to several red spots, confirming partial repression of *ade6*. These data suggest that, although there is a significant pool of hairpin siRNA associated with the RITS complex, it does not guarantee efficient silencing of target genes. Furthermore, Simmer and colleagues confirm that silencing in *trans* depends not only on RNAi factors (Dcr1, Ago1, Arb1 and Rdp1), but also on Clr4, chromodomain proteins Chp1, Swi6 and Chp2, and deacetylases Sir2 and Clr3. These data predict that hairpin-derived siRNA induces heterochromatin formation on target genes, which is also confirmed by the presence of methylated H3 Lys9 and Swi6.

It is interesting that the efficiency of hairpin siRNA-induced *trans*-silencing depends on the position of the target gene (Fig 1). Endogenous *ura4* is a tandem gene and cannot be *trans*-silenced; however, when *ura4* is integrated into *arg3*, *ade6* or *trp1* loci—which are all convergent genes—*trans*-silencing can be partly established (Iida *et al.*, 2008). Furthermore, the integration of *ura4* close to pericentromeric regions makes it a strong target for silencing in *trans*. Convergent genes have been shown previously to result in antisense transcription and transient heterochromatin formation (Gullerova & Proudfoot, 2008), and centromeric repeats are characterized by sense and antisense transcription from convergent promoters, serving as templates for dsRNA synthesis and RNAi-mediated heterochromatin formation. *Trans*-silencing seems to be more efficient on gene targets that are close to heterochromatic regions. Consistent with this idea, antisense transcription has been detected at the *trp1::ura4* locus, which is a strong target for hairpin siRNA-induced silencing (Iida *et al.*, 2008). However, Simmer and colleagues do not detect antisense RNAs at the *arg3::ura4* locus, which

is only partly silenced by hairpin siRNA, suggesting that antisense transcription is not absolutely required for silencing in *trans*, although it enhances the *trans*-silencing effect. Furthermore, close proximity of heterochromatin regions stimulates the *trans*-silencing of target genes.

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Overall, this interesting study by the Allshire lab provides new mechanistic insight about *trans*-silencing in fission yeast, proving the ability of hairpin siRNA to induce the silencing of target genes in wild-type cells. *S. pombe* clearly has the potential to silence genes in *trans*; however, this process seems to be restricted by position and might operate at a co-transcriptional rather than post-transcriptional level.

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Monika Gullerova & Nick J. Proudfoot are at the Sir William Dunn School of Pathology, University of Oxford, UK.

E-mail: nicholas.proudfoot@path.ox.ac.uk

Published online 12 February 2010

EMBO reports (2010) **11**, 145–146.
 doi:10.1038/embor.2010.24