Epigenetic Inheritance and Reprogramming in Plants and Fission Yeast

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Plants and fission yeast exhibit a wealth of epigenetic phenomena, including transposon regulation, heterochromatic silencing, and gene imprinting. They provide excellent model organisms to address the question of how epigenetic information is propagated to daughter cells. We have addressed the questions of establishment, maintenance, and inheritance of heterochromatic silencing using the fission yeast *Schizosaccharomyces pombe* and the plant *Arabidopsis thaliana* by using a variety of genetic and genomic approaches. We present here results showing the cell cycle dependence of RNA in fission yeast RNA interference (RNAi), which is required for proper transcriptional silencing of the centromeric heterochromatin, and that this process occurs during S phase, allowing for precise copying and reestablishment of heterochromatic histone modifications following DNA replication and cell division. We also show that in plants, cells in culture and male germ-line cells undergo massive epigenomic changes correlated with the appearance of a novel class of 21-nucleotide small interfering RNA (siRNA) from transcriptionally reactivated transposable elements (TEs) following loss of heterochromatic DNA and histone methylation. We propose a model for the role of deliberate TE reactivation in germ-line companion cells as part of a developmental mechanism for first revealing and then silencing TEs via small RNA, which may contribute to reprogramming during early development in plants and animals.

Eukaryotic genomes are traditionally characterized as euchromatic or heterochromatic, originally based on their staining patterns (Heitz 1928). These patterns correspond to domains of different chromatin compaction levels, with euchromatin being the less compact of the two. Heterochromatin formation and maintenance have important roles in genome stability and centromere function as well as transcriptional silencing of various TEs and position effect variegation in eukaryotes, X chromosome inactivation in mammals, and gene imprinting in plants and animals (Lippman and Martienssen 2004; Henderson and Jacobsen 2007; Zaratiegui et al. 2007). The two chromatin states also differ in their content and transcriptional activity: Euchromatin is largely composed of genes, whereas heterochromatin is composed of various repeats, including all classes of TEs (Lippman and Martienssen 2004). The difference in composition of the two compartments is reflected in their transcriptional activity: Euchromatin is traditionally viewed as the active portion of the genome, transcribed in response to regulatory cues, whereas heterochromatin is considered transcriptionally silenced, although, paradoxically, it must be transcribed in order to be silenced (Volpe et al. 2002).

The molecular and cytological distinctions of the two chromatin compartments are established by a combination of DNA and histone modifications (Martienssen and Colot 2001; Henderson and Jacobsen 2007). In both plants and fission yeast, heterochromatic DNA is associated with a histone octamer carrying a dimethylation mark at lysine 9 on the amino terminus of histone H3 (H3K9me2). In plants, and *Arabidopsis* in particular, heterochromatic DNA is also heavily methylated, with 5-methyl cytosine (5mC) found in all three possible sequence contexts found in plants: CG, CHG, and CHH (Henderson and Jacobsen 2007). The targeting of heterochromatic DNA and histone

modifications is achieved through the action of RNAi, which provides the sequence specificity necessary to guide the silencing complexes containing DNA and histone modifiers (Zaratiegui et al. 2007).

EPIGENETIC INHERITANCE IN FISSION YEAST

The three centromeres of the fission yeast *S. pombe* have been an excellent system to study the molecular mechanisms that establish heterochromatic silencing and its inheritance upon cell division. Their repetitive structure is very similar, but simplified, compared to higher eukaryotic centromeres (Smith and Corces 1995). The central centromeric region is the site of kinetochore binding that is flanked by innermost repeats which, in turn, are flanked by the outermost repeats consisting of *dg* and *dh* repeat sequences. The *dg* and *dh* repeats are enriched in H3K9me2 (Volpe et al. 2003), which is bound by the fission yeast homolog of the heterochromatic protein 1 (HP1) Swi6 (Holm et al. 2005). This, in turn, is necessary for proper attachments of the kinetochore (Nonaka et al. 2002). Targeting of the heterochromatic chromatin modifications is accomplished by RNAi (Volpe et al. 2002).

RNAi mechanisms take advantage of the *dh* and *dg* transcripts being generated from both DNA strands by RNA polymerase II (PolII) (Volpe et al. 2002). The sole fission yeast Argonaute protein Ago1 cotranscriptionally slices the nascent heterochromatic transcripts (Irvine et al. 2006). This is hypothesized to create sites for the RNA-dependent RNA polymerase (RDRC) complex, which results in formation of double-stranded RNA molecules. These are quickly processed into 21–24-nucleotide siRNA molecules by the Dicer protein Dcr1 (Colmenares et al. 2007). The siRNA molecules then serve as guides when they are bound

by Ago1 and incorporated into the RNA-induced transcriptional silencing (RITS) complex (Verdel et al. 2004). This is required for the final step of heterochromatin formation, whereby the RITS complex interacts, through an unknown mechanism, with the Rik1 complex that contains the histone methyltransferase Clr4 responsible for H3K9me2 methylation (Horn et al. 2005). Ultimately, H3K9me2 is recognized by the chromodomain-containing Swi6, which itself is bound by cohesins (Holm et al. 2005), ensuring the proper functioning of fission yeast centromeres and chromosomal segregation during cell division.

Heterochromatin and the Cell Cycle in Fission Yeast

Given the basic premises of chromatin replication, it is clear that the need for the RNAi-targeted heterochromatin formation process differs between the stages of the cell cycle. It also poses a problem for heterochromatin inheritance, following cell division, because, in the case of fission yeast, the epigenetic information is carried by the histone marks that themselves are not inherited (Kloc and Martienssen 2008).

We have addressed the question of heterochromatin inheritance in fission yeast by profiling *dg* and *dh* transcripts, siRNAs, and histone modifications throughout the cell cycle. Contrary to most eukaryotes, fission yeast heterochromatin is known to replicate during early S phase (Kim and Huberman 2001). This is precisely the time in the cell cycle when the *dg* and *dh* centromeric transcripts can first be detected in cells arrested in early S phase (Kloc et al. 2008), concomitant with the localization of PolII in the same regions (Chen et al. 2008). The *dg* transcripts often appear slightly ahead of the *dh* transcripts, which may be due to the two repeats being located at different distances from the origins of replication. At the same time, H3K9me2 local concentration is at its lowest at the same time in the cell cycle. With the progression of heterochromatic DNA replication past early S phase, an increase in the RNAi factors can be seen, as well as the appearance of heterochromatic siRNA. This indicates that RNAi occurs specifically in the S phase (Kloc et al. 2008). Consequently, this is followed by an increase in H3K9me2, upon the finish of DNA replication, and the faithful reestablishment of heterochromatin. The H3K9me2 levels further increase, peaking during the G₂ phase of the cell cycle.

The loss of Swi6 binding in heterochromatic repeats is the earliest event detected so far that leads to cell-cycle-specific phasing in heterochromatin transcriptional activity, RNAi activity, and histone modification. Swi6 binding depends on the presence of H3K9me2; however, Swi6–H3K9me2 interaction is further dependent on the so-called phosphomethyl switch (Fischle et al. 2003). This term refers to the presence or absence of a phosphate group on histone H3 serine10 (H3S10p), located directly next to the lysine 9 residue carrying the dimethylation modification. In the presence of H3S10p, the Swi6–H3K9me2 interaction is abolished and the transcriptional silencing of the region is lost (Chen et al. 2008; Kloc et al. 2008). Phosphorylation of H3S10 is under the control of the aurora-B kinase Ark1.

Taken together, these results point to a cell-cycle-regulated and coordinated series of molecular events that ensure that epigenetic information contained within parental chromosomes is faithfully transmitted to daughter chromosomes, even in the absence of a directly heritable epigenetic mark such as DNA methylation.

EPIGENETIC INHERITANCE IN PLANTS

Plants offer the full palette of epigenetic modifications, both at the level of DNA methylation and numerous histone modifications. This potential for epigenetic control in plants impacts plant development, responses to biotic and abiotic stress, and integration of environmental and ontogenic cues in the transition to flowering (Henderson and Jacobsen 2007). Perhaps one of the best-studied epigenetic systems in plants is heterochromatin regulation (Lippman and Martienssen 2004).

Heterochromatic Changes in Proliferating Immortalized Plant Cells

Plant cell culture is a known source of induced genetic and epigenetic variation (Hirochika 1993; Kaeppler et al. 2000). A number of plant species when grown in culture undergo transposon reactivation and a number of genes undergo epigenetic changes that lead to epiallele formation (Grandbastien et al. 1989; Kaeppler et al. 2000). This represents a good model for studying the epigenetic mechanisms involved in establishment and maintenance of heterochromatin in a higher eukaryote such as *Arabidopsis*.

We first examined a compendium of gene expression profiles from various organs and cell types from *Arabidopsis*, including a long-term cell suspension culture (Schmid et al. 2005). All of the gene expression profiles contained within this set were created using the Affymetrix ATH1 gene expression platform. This array contains about 1200 specific oligonucleotide probes matching various copies of transposable elements in the *Arabidopsis* genome. This analysis showed that a large number of TEs (mainly those that represent relatively intact copies in the genome) become reactivated in cell suspension culture, whereas TEs remain transcriptionally silenced in *Arabidopsis* leaves and callus samples.

Transcriptional reactivation of TEs in plants is accompanied by changes in epigenetic chromatin modifications. This can be accomplished by down-regulation or by mutations in key regulators of heterochromatin: DNA methyltransferases (*MET1*, *CHROMOMETHYLTRANSFERASE3* [*CMT3*], and *DOMAINS REARRANGED METHYLTRANSFERASE1* and 2 [*DRM1* and *DRM2*], chromatin remodeler *DECREASE IN DNA METHYLATION1* [*DDM1*], histone methyltransferases, in particular the major H3K9me2 methyltransferase KRYPTONITE (KYP), as well as some components of the RNAi pathway (Lippman et al. 2003). The *ddm1* mutation has been particularly well studied in the context of TE regulation, because most TEs in the *Arabidopsis* genome become reactivated in this background (Singer et al. 2001; Lippman et al. 2003). This is accompanied by a nondiscriminating loss of heterochromatic DNA and H3K9me2 methylation (Lippman et al. 2004).

We therefore analyzed the chromatin states of immortalized *Arabidopsis* cells grown in a long-term suspension culture using a custom-made tiling microarray of *Arabidopsis* chromosome 4 to determine the DNA and histone methylation patterns (<http://chromatin.cshl.edu/epiculture/>). As predicted, we detected significant hypomethylation of heterochromatic DNA and loss of H3K9me₂, often replaced by the euchromatic histone modification H3K4me₂. Interestingly, these changes only affected some families of TEs, whereas other TEs, often spatially juxtaposed to the hypomethylated TEs, remained decorated with heterochromatic marks. Our experiments also revealed that these patterns are stable and mitotically heritable throughout many cell divisions. Because loss of heterochromatic marks was specific to some but not all TE families, we postulated that this was not an effect of transcriptional down-regulation of numerous chromatin regulators. Indeed, expression profiling revealed that all of the known chromatin regulators are overexpressed in cell culture, including all classes of DNA and histone methyltransferases and proteins involved in RNAi. The only protein whose expression was lost in cell culture was ROS1, a DNA demethylase (Zhu et al. 2007). We then used next-generation Illumina DNA sequencing to profile small RNA molecules in this sample and compared this profile to the small RNA profiles of young rosette leaves and freshly induced callus samples. This analysis revealed drastic shifts in siRNA abundance and length specific to those TE families that lost heterochromatic marks, with a shift from the predominant 24-nucleotide-size class to the 21-nucleotide size class whose production is under different genetic control (Fig. 1) (Brodersen and Voinnet 2006). This change was particularly striking for the demethylated and reactivated family of *Athila* retrotransposons (Fig 1). It therefore seems that this failure to specifically target appropriate heterochromatic marks upon cell division is due to perturbations in the RNAi systems used to target heterochromatic modifications. However, the fact that the heterochromatic state of other TE families, such as *Atlantys*, is faithfully reproduced over many cell divisions reflects the general availability of the core RNAi machinery and other chromatin regulators. The loss of heterochromatic modification was also detected in short regions of homology with TEs such as short interspersed repetitive elements (SINEs). This can have a particularly significant effect on gene regulation because SINEs are often found in the vicinity of genes. In the case of the imprinted gene *FWA* (Kinoshita et al. 2004), the SINE known to be the imprinting site in planta, becomes hypomethylated in cell culture, with a 25-fold increase in transcription at the *FWA* locus.

Concurrently with the loss of heterochromatic silencing in TEs, we also detected genic hypermethylation in these immortalized plant cells. Although the majority of hypermethylation is located in the coding regions and corresponds to an increase in the novel gene body CpG methylation (Vaughn et al. 2007), a number of genes undergo epiallele formation in these cells. This was evidenced by the concomitant appearance of novel DNA and H3K9me₂ methylation. In addition, close to 90% of these new epialleles, including an epiallele at the *AGAMOUS* (*AG*) locus, a gene known to be prone to epiallele formation (Jacobsen et al. 2000), also have novel 24-nucleotide

Figure 1. Size distribution of siRNA sequences in plant cell suspension culture (A), wild-type (Col) leaves, and wild-type (Col) callus. The relative frequencies of heterochromatic small RNAs (B) and those matching exclusively *Arabidopsis* retrotransposon families *Athila* (C) and *Atlantys* (D) were plotted according to size in cell culture 4 days and 7 days after transfer to fresh medium. The 21-nucleotide siRNAs accumulate only in cultured cells but not wild-type leaf or callus samples and correspond to *Athila* elements (which lose methylation), but not to *Atlantys* elements (which do not). (Modified from Tanurdžić et al. 2008.)

siRNA associated with them in cell culture. It is tempting to speculate that the appearance of novel epialleles in cell culture may be, at least partially, due to the inability of the cells to prune RNA-directed DNA methylation due to transcriptional down-regulation of *ROS1* (Zhu et al. 2007). Intriguingly, the proximal *ROS1* promoter contains a fragment of a helitron TE that becomes hypermethylated in cell culture, and thus, *ROS1* may itself be prone to epiallele formation.

Epigenetic Reprogramming and Plant Germ Cells

In addition to TE reactivation in cell culture, reexamination of previous *Arabidopsis* expression profiling data (Schmid et al. 2005) revealed that the transcriptional reactivation of TEs also occurs in mature wild-type pollen (Slotkin et al. 2009). Unlike animals, plant meiocytes differentiate from adult somatic cells late in development. The pollen grain is produced from two subsequent mitotic divisions that occur after meiosis, so that pollen contains three haploid cells. The largest is the pollen vegetative cell, controlled by the pollen vegetative nucleus, which contains decondensed chromatin. Enclosed within the pollen vegetative cell are two sperm cells with compact chromatin. The vegetative nucleus does not contribute genetic material to the next generation, whereas the two sperm cells are involved in the double fertilization process typical of most seed plants, providing paternal genetic and epigenetic contributions to the zygote and the endosperm.

Our analysis of TE transcriptional activity of pollen using *Arabidopsis* microarray data (Becker et al. 2003; Honys and Twell 2004; Pina et al. 2005; Schmid et al.

2005) showed that although TEs are generally silenced throughout the plant, they are up-regulated in pollen. This co-coordinated TE up-regulation was not dependent on the chromosomal position of TEs nor was it specific to particular families of TEs. Therefore, we concluded that TE transcriptional reactivation in pollen is due to a loss of particular *trans*-acting silencing factors.

Using gene-trap and enhancer-trap reporter lines inserted into TEs (Sundaresan et al. 1995), we assayed the precise location and timing of TE reactivation in the pollen grain. As expected, no GUS staining was detected in the plant body; however, strong GUS staining was detected in mature pollen for all 24 different reporter lines with insertion in various TEs throughout the *Arabidopsis* genome. Furthermore, upon closer inspection, we were able to confirm that the positive GUS-staining signal in pollen was in the pollen grain cytoplasm, whose content is under the control of the vegetative nucleus (VN). In contrast, no GUS accumulation was detected in the sperm cells (SCs). We further tested the hypothesis that TE reactivation is specific to the VN by looking at novel, heritable transposition events using transposon display. We could detect numerous transposition events in the pollen DNA itself, but we were not able to detect any novel transposition in 500 F₁ plants. This evidence also supports the notion that pollen TE reactivation is specific to the VN and does not occur in the SC. In addition, microarray data and reverse transcriptase–polymerase chain reaction (RT-PCR) both demonstrate that TEs are not expressed in SCs purified from the pollen grain using fluorescence-activated cell sorting (FACS) (Borges et al. 2008).

What is the extent, then, of epigenetic changes in the VN chromatin that allow transcriptional TE reactivation? We assayed DNA methylation by sequence analysis of bisulfite-converted DNA from pollen as well as purified SCs using TE-specific primers in PCR amplification. The results of this experiment showed that, on average, TEs are hypomethylated in the genomic DNA isolated from pollen, retaining some DNA methylation. In particular, methylation seems to be lost from the asymmetric methylation sites, which is targeted by siRNA. In contrast, SC DNA contains fully methylated cytosines in both symmetric and asymmetric sequence contexts. This result implicated a VN-specific down-regulation of one or more genes required for heterochromatic silencing in *Arabidopsis* and may be accompanied by active DNA demethylation specifically targeted to the VN. RT-PCR analysis showed that of several candidate genes known to regulate TE and heterochromatic silencing, genes involved in the production of the 24-nucleotide siRNA (particularly *RNA DEPENDENT RNA POLYMERASE2* [*RDR2*] and *DICER-LIKE3* [*DCL3*]) are down-regulated in pollen, whereas the *trans*-acting siRNA and microRNA (miRNA)-generating pathways (21-nucleotide *trans*-acting siRNA and miRNA) seemed to be fully functional. Importantly, we also showed that the master regulator of heterochromatic silencing, DDM1 protein, does not accumulate in the VN but does in the SCs.

We investigated the role of siRNA in targeting heterochromatic silencing in the male germ line by high-throughput sequencing of small RNA molecules from pollen and isolated SCs and compared them to small RNA

data sets from wild-type *Arabidopsis* leaves and inflorescences as well as *ddm1* inflorescences. When the small RNA sequences are aligned to the reference genome, a striking similarity in the patterns and size of matching siRNAs is seen between the two samples with epigenetically misregulated TEs: *ddm1* plants and pollen (Fig. 2). Most TEs, such as *AtMu1*, seem to lose or have significantly reduced levels of the heterochromatic 24-nucleotide siRNA in both *ddm1* (Lippman et al. 2003) and pollen. In addition, the largest family of TEs in the *Arabidopsis* genome, the *Athila* retrotransposons, show a massive increase in the 21-nucleotide siRNA in both samples (Fig. 2). We therefore conclude that upon epigenetic reactivation of TEs, a novel class of siRNA, the 21-nucleotide in size, and matching *Athila* TEs, is massively up-regulated.

Interestingly, the 21-nucleotide siRNA from reactivated TEs accumulate in the SC small RNA library (Fig. 2), even though the primary TE transcripts are not found in the SC RNA. This result would imply that the 21-nucleotide siRNA can move between the VN and SC. We tested whether small RNAs could communicate from the VN to the SC by expressing an artificial miRNA (amiRNA) in the VN (Engel et al. 2005; Schwab et al. 2006) and targeting a green fluorescent protein (GFP)

Figure 2. siRNA distribution according to size, frequency, and sequence homology in *Arabidopsis* pollen, sperm cells, and a *ddm1* mutant. Distributions of 21- and 24-nucleotide siRNAs are shown for a 65-kb section of pericentromeric heterochromatin from chromosome 4. Gene and ORF annotations include DNA transposons (red), retrotransposons (green), and gene (yellow). Bars represent copy-corrected counts per read of 21- and 24-nucleotide siRNAs in 100-bp windows for wild-type inflorescence, wild-type pollen, *ddm1* inflorescence, and wild-type sperm cells. In pericentromeric heterochromatin, large peaks of 21-nucleotide siRNAs in *ddm1*, sperm cells, and pollen match *Athila* family retrotransposons.

Figure 3. Model of TE reactivation and its role in the reprogramming of the germ line. The pollen VN and SCs differ in DDM1 localization and TE expression. Reactivated TEs, such as *Athila* retrotransposons, from the VN produce 21-nucleotide siRNAs via RNAi. This novel class of siRNA is mobile and accumulates in the SC. Thus, TEs are “revealed” by epigenetic reprogramming of the VN in order to target and silence themselves in the SC.

reporter expressed specifically in the SC (Engel et al. 2005). In the plants homozygous for both the amiRNA and GFP reporter, we could detect almost complete silencing of the GFP reporter in the SC. These data further corroborate our hypothesis that small RNA can move between the two nuclear compartments in pollen and communicate with the silencing signal generated in the VN to the SC to ensure TE silencing and both the genetic and epigenetic stability of germ cells (Fig. 3).

SMALL RNA-MEDIATED TRANSPOSON CONTROL IN ANIMALS AND PROTISTS

Our results may shed light on transposon regulation in animals as well as in plant germ cells and even in protists such as *Tetrahymena* and *Paramecium*. In plants and protists, genomic reprogramming seems to be confined to companion nuclei, such as the vegetative nucleus in pollen and the macronucleus in protists, which undergoes DNA elimination and RNAi and does not contribute to the next generation. In mammals, transposon silencing is transiently lost through extensive reprogramming, both in the germ line and in the early embryo. An ancient conserved mechanism of reprogramming may underlie transposon activation in each case.

Recently, there has been a great deal of interest in germ-line small RNA in *Drosophila* and the mouse. Much of this research has focused on Piwi-interacting RNA (piRNA), a 25–29-nucleotide small RNA associated with the PIWI class of Argonaute proteins (which are apparently not found in plants) (Aravin et al. 2007a; Gunawardane et al. 2007). Although their biogenesis is

still somewhat obscure, it depends on the PIWI proteins themselves (Aravin et al. 2007a; Gunawardane et al. 2007). Retrotransposon targeting is achieved by sequence specificity of piRNAs that arise from genomic locations with sequences containing antisense retrotransposon fragments (Aravin et al. 2007b; Brennecke et al. 2007). In the presence of active transposons, sense piRNAs are also found, which are thought to be derived from the transposon, and may help to amplify the more abundant antisense piRNAs (Gunawardane et al. 2007). It is generally assumed that transposons have evolved to infect the germ line, which is where piRNAs mount a host defense (Sarot et al. 2004; Brennecke et al. 2007).

On the basis of our results in plants, however, it is possible that not only are piRNA expressed in the germ line, but so are the transposons they target, deliberately released by the host to provide a small RNA signal and efficiently silence themselves. In *Drosophila*, somatic follicular cells adjacent to female germ-line stem cells are the site of long terminal repeat (LTR) retrotransposon *Gypsy* expression (Sarot et al. 2004; Brennecke et al. 2007). Furthermore, piRNA-mediated repression of hybrid dysgenesis by non-LTR retrotransposons depends on expression of full-length autonomous elements in nurse cell nuclei adjacent to the oocyte (Chambeyron et al. 2008). It is possible that piRNA or TE primary transcripts are generated in these companion cells and then translocated into the oocyte.

In mammals, deep sequencing of small RNA from oocytes has revealed abundant 25–29-nucleotide piRNA and similarly abundant 21-nucleotide endogenous siRNA (Tam et al. 2008; Watanabe et al. 2008). In these reports, the vast majority of 21-nucleotide siRNAs were from retrotransposons, as we have observed in plant sperm cells (Slotkin et al. 2009). It is not yet known at which stage of germ-line development these siRNAs arise; however, the transient loss of heterochromatin in germ cells around E11.5 (Hajkova et al. 2008) could provide the window of opportunity for retrotransposon transcripts and associated 21-nucleotide siRNA to be produced.

REPROGRAMMING AND CANCER

Reprogramming is not limited to the germ cells. Indeed, similarities in expression profiles among germ cells, stem cells, and cancer cells have, in part, led to the “cancer stem cell” hypothesis (Reya et al. 2001), and TEs are significantly up-regulated in these contexts. Cultured cells in general, and especially cancer cells, lose methylation genome-wide, largely from transposons and repeats. In addition, developmental reprogramming itself, in which a handful of transcription factors induce a pluripotent state, is greatly enhanced by inhibiting DNA methylation genome-wide (Meissner et al. 2008; Mikkelsen et al. 2008). In the developing embryo immediately after fertilization, DNA methylation is lost (passively) from the female pronucleus and (actively) from the male pronucleus, only being regained after differentiation of the trophoblast, which remains hypomethylated (Branco et al. 2008). In both the developing oocyte and the developing embryo, these cycles of demethylation and remethylation

depend on down-regulation and mislocalization of the DNA methyltransferases Dnmt1, Dnmt3a, and Dnmt3b, resembling reprogramming in pollen in this respect.

The activation of transposable elements is thus programmed and “intentional” in somatic and germ cells in both plants and animals. Rather than representing a victory of parasite over host, we propose that the transposons are activated transiently at a time when they need to be silenced and that silencing occurs efficiently via RNAi. Importantly, when the RNAi machinery is inactivated in *piwi* mutants in animals or in certain *argonaute* mutants in plants (Nonomura et al. 2007), sterility ensues presumably because of the very significant transposon load. Although certainly speculative, it is hard to ignore the potential for selectively targeting cancer cells by loss of transposon RNAi.

CONCLUSIONS

The genetics and genomics of heterochromatin in fission yeast and plants have expanded our understanding of the establishment, maintenance, and inheritance of epigenetic marks that render heterochromatin transcriptionally silent. Heterochromatin inheritance in fission yeast is tightly coupled to the cell cycle via the phosphomethyl switch, which releases transcriptional repression from heterochromatic repeats. Their transcripts are then rapidly turned over during S phase by RNAi, ensuring that newly synthesized chromatin is once again fully decorated with heterochromatic marks and is returned to transcriptional silencing.

In long-term plant cell culture, similarly to immortalized animal cell lines and cancer cell lines, there is a substantial, yet very localized, loss of heterochromatic silencing specific to families of TEs, in parallel with hypermethylation of gene coding and regulatory regions. The latter represents epigenetic restructuring of the genome in immortalized cells and can lead to mitotically heritable changes in gene expression programs. A novel class of 21-nucleotide siRNA corresponding to the epigenetically reactivated repeats appears in plant cell culture as well as in mutants in *met1* (*Dnmt1*) and *ddm1* (*Lsh1*) and in wild-type pollen.

Down-regulation of chromatin regulators in the pollen VN represents a form of epigenetic reprogramming that leads to reactivation of TEs in companion cells that support the germ cells without contribution to the next generation, revealing the TE content of the parental genome and silencing them in the sperm via mobile siRNA. This likely ensures proper heterochromatic silencing following fertilization.

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