in the region occupied by the germ cells. These cells are already in the endoderm, so are not being moved to the germ cell region by simple repulsion by the mesoderm. This result suggests that Ifitm3 may play another role in germ cell behavior, which localizes germ cells within the endoderm. If so, does it also play a role in the exit from the endoderm, or is this mediated entirely by Ifitm1-mediated repulsion? Loss-of-function analysis of Ifitm3 is badly needed, as is a mechanistic explanation for IFITM function(s) in these processes.

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Replication, Repair, and Reactivation

In a recent issue of Current Biology, Kapoor et al. (2005) and Elmayan et al. (2005) illuminate the linkage between DNA replication and repair and transcriptional gene silencing in plants by showing that mutants in RPA2, a homolog of yeast and mammalian replication protein A, exhibit loss of silencing at transgene loci as well as some transposable elements. This is accomplished by a shift in histone H3 methylation modifications at these loci from a heterochromatic to an euchromatic pattern. Intriguingly, cytosine methylation is unaffected at the reactivated loci, indicating that transmission of DNA methylation and histone modification status can be uncoupled.

Two recent articles in Current Biology (Elmayan et al., 2005; Kapoor et al., 2005) describe a novel component of the transcriptional silencing (TGS) pathway in plants. The surprising discovery made by these two groups brings, in addition to BRU1 (Takeda et al., 2004), FAS1, and FAS2 (Kaya et al., 2001), yet another component of the DNA and chromatin replication and repair machinery into the gene silencing arena. This gene, RPA2, cloned independently by both groups, is an Arabidopsis homolog of the second subunit of the yeast and mammalian replication protein A, which is involved in DNA replication and repair.

The two groups identified rpa2 mutations through genetic screens designed to detect loss of reporter gene TGS. Elmayan et al. (2005) used fast neutron mutagenesis of Arabidopsis plants harboring a transcriptionally silent transgene. This transgene contains 3–4 tandem-repeated copies of pNos::nptII and 35S::GUS reporter genes and is known as L5. In contrast, Kapoor et al. (2005) identified insertional mutants in RPA2 via T-DNA mutagenesis of a population of 20,000 ros1 plants followed by screening for the loss of TGS of a complex reporter transgene containing the LUC reporter gene driven by the RD29A promoter and a nptII gene driven by the 35S promoter. In this case, TGS of the reporter is caused by the loss of functional ROS1, a DNA glycosylase/ligase, in the ros1 background. In both cases, the silent reporter gene driven by the 35S promoter was activated by the rpa2 mutation, while Kapoor et al. report that the RD29A::LUC transgene remained silenced and methylated.

How is TGS lost in rpa2 mutants? Interestingly, this mutation does not seem to affect some of the usual suspects in TGS: namely, promoter cytosine methylation and siRNA production. It does, however, affect histone H3 methylation patterns. While the 35S promoter in wild-type plants in silenced via Histone H3 lysine-9 methylation (H3mK9), in rpa2 mutants this is replaced by H3mK4, an epigenetic mark characteristic of active chromatin. This was sufficient to activate reporter gene transcription even in the presence of cytosine methylation.

Furthermore, RPA2 is needed for more than just 35S promoter silencing. Elmayan et al. and Kapoor et al. report that the loss of RPA2 can lead to reactivation of typically heterochromatic Athila, AtMu1 and AtLINE1-4 transposable elements (TE). As was the case with the reporter genes, TE reactivation was not accompanied by changes in DNA methylation or siRNA, with both remaining unchanged in the mutant. In addition to the molecular phenotypes, rpa2 plants exhibit shorter stature and early flowering when compared to the wild-type plants and, like ros1 plants, are also more sensitive to DNA-damaging agents.

Together, the papers by Kapoor et al. and Elmayan et al. indicate that, while the mechanisms for propagation of DNA and histone methylation are physically coupled to replication and repair, they can be uncoupled from...
one another, a fact made more intriguing by the observation that ROS1 and RPA2 interact physically. DNA methylation may be recruited along with H3mK9, but once the heterochromatic state is established, H3mK9 is transmitted in a process mediated by RPA2. That transmission of DNA and histone methylation would be uncoupled is not entirely surprising since many eukaryotes do not utilize DNA methylation as a regulatory engine, while H3mK9 is more widely conserved.

What might the mechanism be? Kapoor et al. suggest that RPA2 might be involved in heterochromatic spreading, such that histone modifications associated with the RD29A::LUC gene might spread via RPA2 to the 3SS::NPT gene. While an attractive idea, 3SS promoter silencing occurs in other contexts besides the RD29A::LUC construct (Bender, 2004). In these cases, spreading is a less likely explanation. An alternative hypothesis is that heterochromatic silencing of native promoters via DNA methylation may be explicitly suppressed in Arabidopsis. In wild-type plants, small RNAs may direct methylation and silencing of the RD29A transgene and some native promoters, but this is reversed by a functional ROS1. These promoters might activate or recruit ROS1 and DEMETER via interactions with proteins or RNA, which serve to distinguish them from coding sequences. This is consistent with the findings that ROS1 appears to suppress methylation predominantly at predicted transcription factor binding sites within the RD29A promoter but ros1 mutants have no general excess of DNA methylation. The viral 3SS promoter is not a native promoter and may thus be missing transcription factor binding sites required to stimulate the demethylation machinery. Thus, when DNA methylation occurs at 3SS promoters, it is not removed and the promoter remains methylated. In the case of FWA, a gene whose promoter is regulated by DEMETER, methylation is recruited in the first place to an upstream transposon by siRNA (Kinoshita et al., 2004; Lippman et al., 2004).

Different classes of transposable elements (TE) respond differentially in silencing mutants (Lippman et al., 2003), indicating that different TGS mechanisms control subsets of TEs. In comparison to met1 and ddm1, only a very small proportion of Athila retroelements are reactivated in rpa2 (Elmeyan et al., 2005), although activation of the DNA class transposon AtMu1 may be more substantial, but in each case methylation is unchanged (Kapoor et al., 2005). Because these TEs utilize different replication and integration strategies and yet can reactivate in rpa2, one attractive hypothesis to explain these results is that RPA2 facilitates silencing only of recently integrated TEs which might trigger the repair pathway if some level of transposition activity remained.

In yeast, components of the origin recognition complex recruit silencing factors, suggesting a role for DNA replication in heterochromatin, but RPA homologs have not been implicated (Loo and Rine, 1995; Suter et al., 2004). These papers describe the first example of such a role for RPA proteins in a higher eukaryote.

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Selected Reading