ments to water (*3*). Second, there is a preferred curvature (the monolayer spontaneous curvature) that depends on the lipid composition. This curvature competes with a free-energy cost for the creation of density variations in the volume of the hydrocarbon chains (the hydrocarbon void cost).

Consideration of this competition between curvature and the hydrocarbon void cost has provided great insight into the energetics of lipid phase transitions, in which bilayers fuse into bulk phases with completely different structures. For example, the H_{II} phase consists of hexagonal arrays of water-cored lipid monolayer tubes (*4*). X-ray diffraction measurements have elucidated the structure and dimensions of the bilayer and H_{II} phases on either side of the phase transitions. But the absence of periodic, stable lipid phases of fusion structures has impeded progress in understanding the fusion intermediates.

Early calculations of the free energy of the stalk structure suggested that the cost of bending the lipid monolayers into the tight curvatures at the junction point was impossibly high, up to hundreds of times the thermal energy scale. This is known as the energy crisis of membrane fusion. Later, it was realized that the exact shape of the junction has an enormous effect on the curvature energy and that small changes in the detailed shape could bring the energy of the junction to a realistic level (*5*).

A second problem has been the limited understanding of the magnitude and functional form of the free-energy cost of low-density voids in the hydrocarbon chains at the stalk junction. Again, changes in the exact structure of the junction point, when combined with various assumptions about the form of the hydrocarbon void cost, lead to tractable free energies in model studies (*6*).

There were, however, no experimental measurements of the shape and density variations of the fusion intermediates to validate the models. This is exactly what Yang and Huang provide. The authors show that in the presence of small amounts of water, certain diphytanoyl phospholipids found in archaebacteria form a stable phase consisting of a three-dimensional hexagonal array of stalk-like structures. This stalk-like phase is bounded at high water content by a phase of stable bilayers and at low water content by an H_{II} phase. The stalk-like phase was sufficiently well ordered that Yang and Huang could determine its low-resolution three-dimensional structure.

The experimental system of Yang and Huang allows an analysis similar to that used to analyze the bilayer-H_{II} phase transition (*4*). It should help to answer many open questions: What are the curvature energies of the observed stalks? How do their structures vary with temperature, lipid composition, and the concentration of divalent cations such as Ca^{2+}? How do oils, which greatly affect the ability to form low-density hydrocarbon voids, change the structure and stability of the stalk phase? Can a stalk phase be made that incorporates fusion proteins? Answers to these questions will improve our understanding of one of the most important processes in cellular systems.

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PERSPECTIVES: MOLECULAR BIOLOGY

RNAi and Heterochromatin—a Hushed-Up Affair

Robin Allshire

The highly repetitive DNA (heterochromatin) of eukaryotic genomes contains a large number of repeats and transposons. Regions of heterochromatin are frequently associated with centromeres, which are crucial for the segregation of chromosomes during cell division. Transgenes inserted into heterochromatin domains can be shut down through the influence of silent chromatin in this region. The formation of silent chromatin requires that histone H3 of chromatin be deacetylated and then methylated on lysine 9. The methylated lysine 9 residue binds to heterochromatin protein 1 (Swi6 in fission yeast), leading to a block in transcription. Subsequent methylation of the DNA in this region then locks the chromatin into the silent state (*1*). Genes can also be silenced at the RNA level by RNA interference (RNAi), which depends on the accidental or deliberate expression of double-stranded RNAs (dsRNAs). These dsRNAs are processed and amplified into small interfering RNAs (siNAs) that bind to and degrade any mRNA transcripts with the same sequence, resulting in loss of expression of the genes encoding these mRNAs (*2*). Although seemingly separate mechanisms, H3 lysine 9 methylation and RNAi were recently found to be part of the same gene-silencing pathway in the fission yeast *Schizosaccharomyces pombe*. This unexpected discovery is providing new insights into how different forms of chromatin silencing may be triggered.

In the fission yeast, the repetitive DNA at centromeres is maintained in a transcriptionally silent state by methylation of H3 lysine 9 and binding of Swi6 to the modified chromatin. Swi6 is itself specifically required for cohesion between sister chromatids at centromeres. On page 1833 of this issue, Volpe *et al.* (*3*) reveal the surprising finding that deletion of genes encoding components of the RNAi pathway in fission yeast leads to loss of gene silencing. Deletion of Argonaute, Dicer, or RNA-dependent RNA polymerase (Rdp1) alleviated silencing of transgenes inserted into the centromeric heterochromatin of *S. pombe*. Loss of gene silencing was not an indirect effect because intact RNA transcripts transcribed from the centromeric repeats were detected in yeast cells lacking RNAi or Swi6, and Rdp1 was still found associated with centromeric heterochromatin. The upshot is that methylation of H3 lysine 9 and binding of Swi6 is abrogated in the *S. pombe* mutants lacking various components of the RNAi machinery, resulting in activation of the transgenes. Notably, Argonaute, Dicer, and Rdp1 are absent from budding yeast, which relies on the Sir2, Sir3, and Sir4 proteins to provide a distinct form of chromatin-based silencing.

A link between dsRNAs and chromatin silencing has also been suggested by observations in plants: A plant transgene can be silenced by expression of dsRNAs with homology to part of that transgene, resulting in methylation of the homologous DNA region (*4, 5*). So, how does the RNAi machinery contribute to assembly of silent chromatin? One possibility is that methyltransferases (the enzymes that methylate DNA and histones) containing chromodomains might be recruited to target gene loci by processed dsRNAs, triggering the formation of silent chromatin. A precedent for this is the targeting of an acetyltransferase containing an RNA binding chromodomain, which acetylates lysine 16 of histone H4, to the X chromosome of male *Drosophila* by a dsRNA (*6*).

In their new study, Volpe *et al.* propose a model in which dsRNAs derived from centromeric heterochromatin repeats are pro-

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cessed into siRNAs at the site where they are transcribed (see the figure). Indeed, siRNAs homologous to centromeric repeats have been detected in wild-type fission yeast in a region where both top and bottom DNA strands are transcribed (*7*). These processed dsRNAs may recruit the Clr4 histone H3 methylase via its chromodomain, resulting in methylation of H3 lysine 9 in adjacent chromatin. This would enable Swi6 to bind to the chromatin, with subsequent gene silencing, and inhibition of transcription from the top strand of the centromeric repeat (see the figure). Such a pathway would provide a fail-safe system in which the bottom strand is constitutively transcribed, with breakthrough transcription of the top strand allowing formation of dsRNAs that trigger chromatin silencing and hence repression of production of the top transcript. Lateral spread of dsRNA processing, noted in some systems [(*8*) and references therein], may allow brief synthesis of more extensive dsRNAs (and thus siRNAs) and subsequent silencing of a larger region of chromatin. It is possible that dsRNAs can act to silence homologous DNA sequences at other loci, but this raises the question of how they seek out such targets. It also remains to be seen whether chromatin-associated or chromatin-modifying factors are in fact directly recruited by these centromeric siRNAs (and, if so, which ones). Other mechanisms cannot be ruled out: It is possible that siRNAs are merely cofactors that activate nearby chromatin-modifying enzymes that have been recruited to the DNA by other means. Indeed, the centromeric siRNAs of *S. pombe* may be completely passive, having been generated simply as a consequence of dsRNA processing.

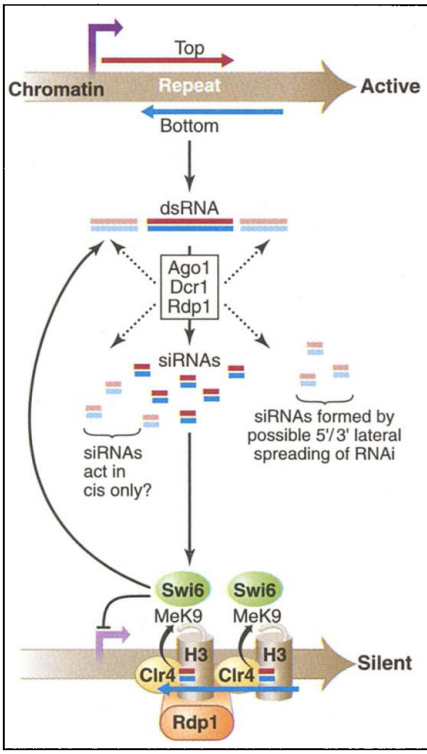
Related mechanisms of gene silencing may operate in mammalian cells, for example, during inactivation of the X chromosome in female cells. The earliest detectable event is expression of *Xist* RNA, which eventually coats the entire X chromosome, followed soon after by H3 lysine 9 methylation, gene inactivation, and ultimately methylation of the DNA (*9*). The mouse Eed protein (a component of the repressive Polycomb complex) is required for inactivation of the paternal X chromosome in extraembryonic tissues (*10*). Moreover, both Eed and Enx1 Polycomb group proteins associate with the inactive X chromosome and thus may be recruited by *Xist* RNA (*11*). However, even though an overlapping transcript (*Tsix*) is produced from the opposite DNA strand, there is nothing to suggest that *Xist* RNA is processed by the RNAi machinery. Other observations indicate that RNA may be involved in the formation of mouse centromeric heterochromatin because lysine 9–methylated H3 cannot be detected in centromeres after ribonuclease treatment of permeabilized mouse cells (*12*).

In addition, RNA transcripts homologous to both strands of centromeric satellite repeats have been reported in mouse cells (*13*).

With an explosion in the use of RNAi as a high-throughput tool for nullifying gene function in various organisms, it is surprising that no attempt has been made, other than in plants (*4, 5*), to explore changes in chromatin structure or modifications at the locus of the affected gene. Recent observations suggest that Polycomb group proteins in the worm *Caenorhabditis elegans* may be important for RNAi-mediated gene silencing (*14*). It is imperative to determine whether the expression of dsRNAs triggers heritable chromatin-based gene silencing at the locus homologous to the effector dsRNAs.

RNAi-dependent chromatin silencing may have originated from mechanisms put in place to immobilize transposable elements and to silence RNA viruses. In *Drosophila* (*15*) and *C. elegans* (*16*), mutation of RNAi components results in transposon activation. Furthermore, the *mut7* mutants of *C. elegans* exhibit a mutator phenotype due to transposon mobilization. The centromeric repeats in fission yeast bind to the Abp1 and Cbh1 proteins, which are also required for the formation of silent chromatin on these repeats (*17*). Abp1 and Cbh1 are homologs of mammalian CENP-B proteins and are related to the transposases found in the *pogo* transposon superfamily. It seems plausible that these centromeric repeats are remnants of ancient transposons, with the Abp1 and Cbh1 binding sites marking positions of the original long terminal repeats of these transposons. Devising methods to silence transposons is one way to deal with these potentially harmful agents, but their accumulation at centromeres apparently has been exploited to ensure efficient cohesion between sister chromatids during cell division (*18, 19*).

The discovery of direct links among centromeric RNAs, RNAi components, and heterochromatin formation in fission yeast immediately changes the way we think about the assembly of different types of silent chromatin. The idea that chromatin can be silenced by RNAi is now firmly established. We eagerly anticipate propagation of this idea and the inevitable deluge of studies required to dissect this process.



Keeping chromatin quiet. Top- and bottom-strand RNA transcripts from the outer centromeric DNA repeats of *S. pombe*. These overlapping RNAs form dsRNAs, which are diced and processed by Argonaute (Ago1), Dicer (Dcr1), and RNA-dependent RNA polymerase (Rdp1) into siRNAs capable of silencing genes. The siRNAs may activate or recruit chromatin-modifying enzymes that promote methylation of lysine 9 in histone H3, allowing the binding of Swi6 to chromatin and the formation of silenced chromatin. This results in repression of top-strand synthesis, although bottom-strand transcription and processing still persist in silent chromatin. Stochastic loss of gene silencing results in production of the top-strand RNA, which immediately anneals to the bottom-strand RNA. This provides dsRNAs for amplification by Rdp1 and cleavage by Dcr1, resulting in regeneration of siRNAs. Annealing of siRNAs via the Ago1/RISC complex may allow Rdp1 to transiently produce more extensive dsRNAs (and thus siRNAs) by lateral spreading both upstream and downstream of where the dsRNAs predominate. This would result in chromatin modifications that spread outward from the region where Rdp1 is associated with chromatin and where abundant siRNAs are generated [see (*8*)].

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Published online 22 August 2002;
10.1126/science. 1075874.
Include this information when citing this paper.