plained by short-range mobilization and recrystallization of the detrital gold particles during postdepositional deformation and heating of the rocks (*1*). This picture is supported by rare samples in which two types of gold particles are found together on a millimeter scale (see the figure) (*9*): one displaying morphological features that are typical of alluvial, windblown, detrital gold (left panel), and the other occurring as irregular intergrowths of minute, well-shaped hydrothermal precipitates (right panel). The detrital particles are unusual in Witwatersrand ore.

The results of Kirk *et al.* (*3*) confirm that the Witwatersrand gold deposits represent Late Archean placers (ancient detrital sediments transported by a river that contain economic quantities of a valuable material). Furthermore, they provide a possible explanation for the extraordinary size of these deposits. Comparison with the amount of gold extracted from other, younger terrains (*10*) suggests an almost exponential decline in the extraction of gold from the mantle into the crust over geological time.

If this postulated decline in gold extraction into the crust is correct, the uniqueness of the Witwatersrand gold province can be explained by three factors. First, the sediments derive from some of the oldest rocks known on Earth. Second, repeated reworking of sediment led to progressively higher gold grades along degradation and deflation surfaces. Third, the gold-bearing sediments escaped from destruction by later mountain-building processes and/or erosion.

Apart from an obvious application in future exploration strategies for Witwatersrand-type gold deposits elsewhere, the findings of Kirk *et al.* (*3*) also have a bearing on our understanding of the early evolution of Earth's atmosphere. Controversy has existed regarding the oxidation potential of the Archean atmosphere (*11*, *12*). Confirmation of a placer origin not only of the gold but also of the associated pyrite and uraninite implies an overall reducing atmosphere during the Late Archean


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**PERSPECTIVES: MEMBRANE FUSION**

# Caught in the Act

**Sol M. Gruner**

Membranes divide eukaryotic cells into numerous compartments in which different chemical processes can take place. On page 1877 of this issue, Yang and Huang (*1*) investigate one of the most fundamental processes involving such membranes. They show how membrane fusion intermediates can be stabilized, allowing detailed structural characterization.

The development of the cellular membrane was a defining advance in the evolution of life. Nature's elegant solution to the constraints on living membranes is the lipid bilayer, a structure that is impermeable to most ions and water-soluble molecules, yet flexible, robust, and able to grow and to heal punctures.

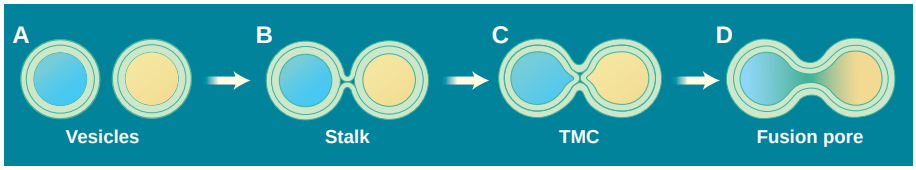
Yet these same properties present problems, because all cells at times need to fuse one membrane compartment with another to mix the contents or deliver new patches of membrane. Scientists have long struggled to understand how bilayers, which seem to be thermodynamically designed to maintain their integrity, can be coerced to fuse and merge with one another.

The primary experimental difficulty in studying this process has been the unstable and highly transient nature of intermediate fusion structures. Yang and Huang (*1*) present an important new experimental tool for studying the fusion process, namely, a stable lattice of lipid fusion structures.

In living systems, fusion is mediated by complex protein machinery. But under appropriate conditions, pure lipid bilayer vesicles can also fuse. It is widely believed that the protein apparatus serves to position the membranes and promote the structural rearrangements that also occur in pure lipid systems, albeit with less control and specificity (*2*). Thus, an understanding of the steps involved in pure lipid bilayer fusion is fundamental to a deeper understanding of the fusion process.

Liquid-crystalline lipid bilayers consist of two opposed monolayer leaflets of highly water-insoluble polar lipids. Within each monolayer, lipid molecules diffuse freely as a two-dimensional fluid. Isolated bilayer vesicles can be brought into close contact—for example, by divalent cations, an electrical pulse, or the addition of hydrophilic polymers. By incorporating fluorescent lipid markers into the bilayers of some vesicles and fluorescent quenchers in others, the mixing of the lipid bilayers upon vesicle fusion can be traced. Similarly, introduction of aqueous fluorescent markers into the aqueous compartment of some vesicles and quenchers into others allows the mixing of the vesicle contents to be monitored.

Such experiments have shown that during fusion, the outer lipid monolayer leaflets exchange molecules before the aqueous compartments. The initial event is the formation of a stalk from locally fused outer leaflets (see panel B in the figure). The radius of the stalk is thought to expand, allowing the inner leaflets to come into contact at the fusion point. This process results in the formation of a transmonolayer contact (panel C) and, upon fusion of the inner monolayers, in a fusion pore (panel D).




**Schematic cross section of the fusion of two lipid bilayer vesicles.** The vesicle bilayers are schematized as two opposed lipid monolayer leaflets. (**A**) Distinct vesicles. (**B**) The outer leaflets fuse into a connecting stalk. (**C**) The stalk radius widens until the inner leaflets touch in trans-monolayer contact (TMC). (**D**) A fusion pore forms.

The challenge has been to understand the energetics of this process. What are the free-energy barriers to fusion of the inner and outer monolayers? And how stable are the various intermediate structures?

Three factors come into play. First, there is a well-understood, steep free-energy cost of exposing lipid hydrocarbon chain seg-

The author is in the Physics Department and the Cornell High Energy Synchrotron Source (CHESS), Cornell University, Ithaca, NY 14853–2501, USA. E-mail: smg26@cornell.edu

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-

The author is at the Wellcome Trust Centre for Cell Biology, Institute for Cell and Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, UK. E-mail: robin.allshire@ed.ac.uk