coupled with oxidative polymerization of specific monomers such as substituted pyrroles. These reactions yield a new type of layered nanocomposite in which sheets of polymers alternate with the host layers (*10*). Other host lattices (silicates, MPS_3) are also able to take up polyethyleneoxide, probably by a solvation mechanism (*11*, *12*). Tetrathiafulvalene, a fundamental molecule in the field of organic conductors, and some related species intercalate layered host lattices [FeOCl (*13*), $FePS_3$ (*14*)]; the guest species organize themselves as stacks that exhibit mixed valency and metallic conductivity. Other examples can be found in a recent review by O'Hare (*15*). Much remains to be discovered; in particular, the host lattice might prevent Peierls distortions from occurring and therefore favor superconductivity.

What is the future for layered nanocomposites? A number of exciting tracks are to be followed, which resemble what might be called two-dimensional supramolecular chemistry (*16*). One track is to combine on the molecular scale the mineral lattice and the organic species, with the hope that some synergies will occur. The possibility of such an effect is demonstrated by Lacroix *et al.* (*1*). The $Mn_{0.86}PS_3(DAMS)_{0.28}$ hybrid organic-inorganic composite actually becomes a magnet below about 40 K because of the spins of the Mn(II) cations of the host lattice (DAMS stands for the hyperpolarizable cationic chromophore 4'-dimethylamino-*N*-methylstilbazolium). This composite therefore associates both strong spontaneous second-order NLO properties and spontaneous magnetization up to quite high temperature by the standards of insulating transparent magnets (*17*). Therefore, the inorganic component provides the magnetic property, the organic chromophore provides the strong hyperpolarizability, and the synergy effect is represented by the orientational influence exerted by the host lattice on the guest organic species. This discovery opens a new avenue of research in the field of "multi-property" materials. Intercalation therefore provides an alternative to sol-gel methods for the synthesis of hybrid nanocomposites, at least in certain circumstances, bringing the advantage of a better crystallinity, a factor which can be of crucial importance when cooperative interactions are required.

One important condition for the future of intercalation is to carry out work at the frontier between organic and inorganic chemistry and to merge properties. If electronic devices operating at a molecular scale are to be developed in the future, it seems reasonable to think that the layered compounds will have a role to play as workbenches to tie down the molecular units. One can imagine x-rays or electron beams writing information by inducing some transformation on a molecule grafted on the surface of a layered material, which would be read by a scanning tunneling or atomic force microscope.

To be interesting, intercalation must not remain confined to laboratories specialized only in solid-state chemistry. It is by itself only a strategy to bring two or more components into intimate contact. It requires that several fields merge at many stages; this begins with, at the synthetic stage, organic and inorganic chemistry and materials science. Then, depending on what works, effort can turn to nonlinear optics, magnetism, energy migration, or photochemistry, and so on to other domains usually well separated. From this range of scientific endeavor, we see that intercalation chemistry is a multidisciplinary field, the development of which requires collaboration on a large scale.

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Remodeling Schemes of Intracellular Pathogens

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The successful microbial pathogen enters a specific host, finds a unique cellular niche, and multiplies even in the face of formidable host defenses (*1*). Many pathogenic bacteria are content to fight their way to the mucosal surface to multiply. Others, such as *Rickettsia* (causative agents of typhus and other arthropod-borne diseases) and *Chlamydia* (causative agents of trachoma and sexually transmitted disease), are obligate intracellular pathogens. To establish a successful infection, these organisms must invade (enter) a eukaryotic cell. The route of cell entry can have a profound influence on host-pathogen interactions hours and even days later in the infectious process. Although we know little about the process of entry, it is clear that microbes exploit normal internalization mechanisms of the host cell (*2*).

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Most microbes that infect humans or that make up their normal flora are held at bay by innate physical and biochemical defense barriers. The microbe breaching these initial obstacles may meet strategically placed phagocytic cells whose major function is to destroy infectious agents. Almost perversely, some microorganisms take up residence in phagocytic macrophages. Among these are the bacteria that cause typhoid (*Salmonella typhi*), Legionnaire's disease (*Legionella pneumophila*), and tuberculosis (*Mycobacterium tuberculosis*), as well as several protozoan pathogens.

The macrophage presents unique advantages and disadvantages to the entering pathogen. It is a long-lived cell and thus provides a potential long-term habitat for the bacterial invader. In addition, because the macrophage serves as an antigen-presenting cell, it may offer the pathogen an opportunity to manipulate the immune system to its own advantage; indeed, survival and replication within the macrophage may even facilitate the journey of the pathogen to a preferred environmental niche. The route of entry into the macrophage is

still unclear, although several cellular receptors have been implicated (*2*, *3*) and several bacterial determinants suggested (*4*). By whatever means, the pathogens do get in!

The major problem the would-be macrophage dweller faces is perhaps not how to get in, but how to survive once inside. The macrophage is equipped with an array of antibacterial activities such as defensins and reactive oxygen and nitrogen intermediates—all of which contribute to a hostile environment. The entering pathogen is encased in a membrane-bound phagosome in which the pH begins to drop, presumably because of the incorporation of a proton–adenosine triphosphatase (ATPase) into the phagosomal membrane. Fusion with lysosomes results in the formation of an acidic (pH <5.0) phagolysosome containing a wide range of activated acid hydrolases and other lethal enzymes.

How do pathogens squelch these potentially lethal host defense mechanisms so that they can proceed with their own agenda of intracellular replication and, in some cases, long-term persistence within the cells? There is more than one way to skin a macrophage. The bacterium responsible for Q fever, *Coxiella burnetti*, not only resists the effect of hydrolytic enzymes, but actually requires an acidic environment for growth (*5*). Other bacteria, including *Listeria monocytogenes* (a causative agent of meningitis and sepsis), *Shigellae*, and some *Rickettsiae*, escape from the phagosome into the cytoplasm (*2*). These pathogens synthesize a phospholipase that dissolves the phagosomal membrane and permits the bacteria to replicate within the host cell cytoplasm.

Still other bacteria, such as *L. pneumophila*, *Salmonella spp.*, and several *Mycobacteriae*, reside within a macrophage vacuole that they remodel into a more hospitable environment. These vacuoles are defined by the unique combinations of cell membrane markers that they bear. For example, major histocompatibility complex class II molecules are selectively excluded from the *L. pneumophila* phagosomes, although other membrane markers are present (*3*). These remodeled phagosomes do not fuse with lysosomes and therefore do not acidify, thus providing a safe haven for the bacteria. In addition, mitochondria and ribosomes are recruited to the phagosome periphery and play some unknown role in the intravacuolar growth of the bacteria (*6*).

In contrast, *Salmonella spp.* reside in two different macrophage vacuolar compartments: one characterized by normal phagolysosomal fusion, which leads to microbial inhibition, and another more "spacious" vacuole that is modified by the bacteria, possibly for replication (*7*). How the bacteria do this is not known. The spacious vacuoles have been shown to contain lysosome-associated membrane markers normally found in the phagosome, although delivery of the late lysosomal marker cathepsin L is delayed.

The most infamous intracellular bacterial pathogen, *M. tuberculosis*, has been shown to reside in a vacuole defective in acidification (*8*, *9*). It has therefore been assumed that there is little interaction between *Mycobacterium*-containing phagosomes and the normal cellular endocytic pathway. A paper by Sturgill-Koszycki *et al.* (*10*) in this issue of *Science* now provides evidence to the contrary. The work not only demonstrates an interaction between the cell's endosomal network and the mycobacterial vacuole, but also suggests a mechanism by which the pathogen may protect itself in the intracellular milieu.

The authors show that the lysosomal-endosomal marker LAMP-1 is present in the mycobacterial vacuole. Yet, in agreement with previous studies, they show that the vacuole is less acidic than lysosomal compartments (pH 6.3 to 6.5 versus <5.5). Sturgill-Koszycki *et al.* also provide evidence that the *Mycobacterium*-containing phagosomes are devoid of the host cell proton-ATPase. In most animal cells, all ingested materials rapidly reach acidic compartments, and this acidification is thought to be mediated by specific vacuolar proton pumps (such as the proton-ATPase) that may be part of the lysosome (*11*). Thus, we are confronted with a situation in which one lysosomal component is present on the mycobacterial vacuole and another is absent. The authors also show that LAMP-1 is maintained continually throughout infection, despite the fact that membrane vesicles containing a secreted mycobacterial cell wall component, lipoarabinomannan, pinch off the vacuole (see figure).

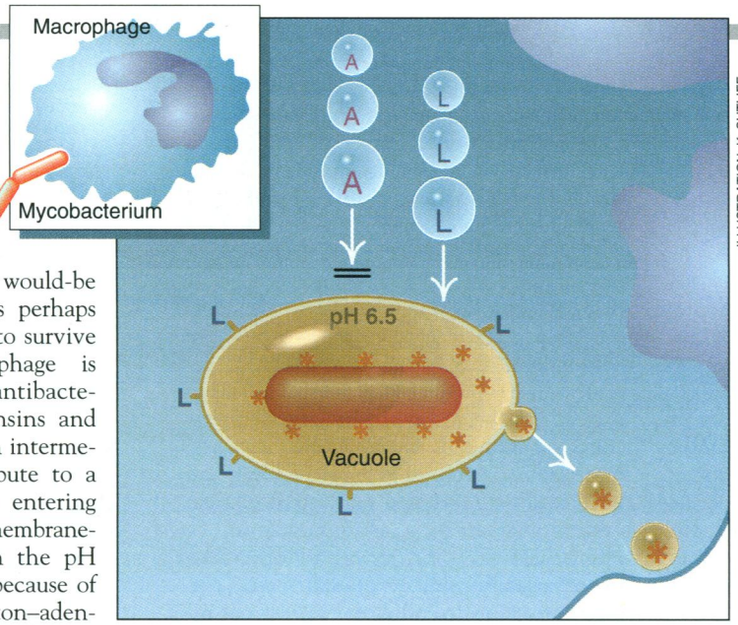

ILLUSTRATION: K. SUTLIFF

Mycobacterial remodeling of a phagosome. After the mycobacterium enters the macrophage, it is maintained within a specialized vacuole that contains the endosomal-lysosomal marker LAMP-1 (L) but is devoid of the proton-ATPase (A). The intravacuolar pH is maintained at 6.5. Sturgill-Koszycki *et al.* (*10*) propose that the mycobacterium selectively inhibits fusion of the vacuole with membrane vesicles containing the proton-ATPase, which may be involved in phagosomal acidification. LAMP-1 is maintained in the vacuole throughout the infection despite the fact that a secreted mycobacterial cell wall constituent, lipoarabinomannan (*), is transported out of the macrophage in dense granules.

Little is known about the exact delivery system of proton-ATPase complexes in the normal cell, and the *Mycobacterium* infection model may prove useful in dissecting this aspect of normal host cell function. Sturgill-Koszycki *et al.* propose that the *Mycobacterium* enters in a vacuole that selectively incorporates vesicles containing LAMP-1 and selectively excludes those containing the proton-ATPase. This possibility implies that there are separate LAMP-1 and proton-ATPase vesicles in the normal endocytic pathway of the cell. Alternatively, the mycobacterial vacuole may fuse with an endocytic vesicle bearing both markers and selectively exclude the proton-ATPase. Reconsideration of experiments by Armstrong and Hart (*12*) may be helpful here. These authors observed *M. tuberculosis* in lysosome-associated vesicles when bacterial entry was mediated by Fc receptors, but not when it was mediated by CR3 receptors. Is it possible that vacuoles formed after entry by Fc receptors contain the proton-ATPase? Is it also possible that later in infection the bacterium uses a second adaptation to escape from the membrane altogether (*13*)?

In the context of human infection, *M. tuberculosis* enters the lung in aerosolized droplets, where it is phagocytosed by resident alveolar macrophages. About 95% of the time, the infection is resolved without overt damage, although the microbe can persist for decades in microscopic granulomatous lesions. Should normal host cellular immunity wane because of age or a compromising disease such as acquired immune deficiency syndrome, then reactivation of the disease can occur. Thus, dissection of the mechanisms of persistence of this organism, as illustrated by the work of Sturgill-Koszycki *et al.*, is central to our understanding of the biology of the host-

pathogen interaction and of the evolution of tuberculous disease.

Many questions about the intracellular lifestyle of M. *tuberculosis* remain to be answered. Which bacterial gene products are involved in the remodeling of the phagosome? How are the intravacuolar bacteria presented to T cells to elicit cell-mediated immunity? What are the implications of preventing vacuolar acidification for the immune response to M. *tuberculosis* infection? If we can answer these questions, we will not only have a better understanding of microbial persistence and normal intracellular trafficking, but we may also have a key to preventing one of the most insidious causes of human morbidity and mortality.

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DNA Replication Origins in Animal Cells: A Question of Context?

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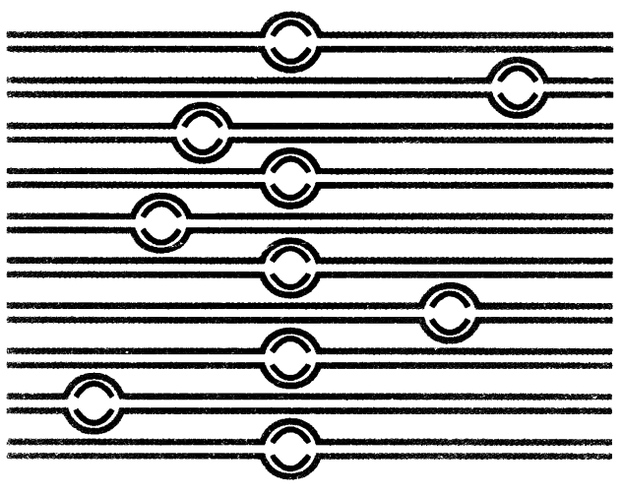
The process of DNA replication, which occurs during each cell division, starts at "origins," places in DNA molecules where the synthesis of a new DNA strand begins. In simpler genomes such as prokaryotes and the budding yeast *Saccharomyces cerevisiae*, replication origins require well-defined sequences called "replicators" (*1–5*) and their interaction with a specific initiator protein complex (*2, 6*). How DNA duplicates itself in more complex metazoan (animal) cells has not been as clear. The field is still pondering whether the replication origins of animal cells correspond to specific nucleotide sequences and, if they do, what those sequences are and how they function.

Until recently, replicators [cis-acting sequences essential for origin function that are identified genetically] had been demonstrated for just two animal cell origins of replication. In one case several short (several hundred base pairs), partially redundant, cis-acting sequences are needed for DNA synthesis at an origin used for amplification of chorion genes in the third chromosome of the fruit fly, *Drosophila melanogaster* (*7*). In the second case, a replicator responsible for an origin of replication downstream of the dihydrofolate reductase (DHFR) gene in CHO (Chinese hamster ovary) cells has been localized within 16 kilobase pairs (*8*). Now a third example of an animal cell replicator has been provided in a recently published study of DNA replication in the human β-globin gene region (*9*). A replication origin normally located just upstream of the β-globin gene is removed by a naturally occurring deletion of this locus.

These three examples convincingly demonstrate the existence of replicators in animal cells. It is likely that most other animal origins will also prove to be associated with replicators. This view is reinforced by the results of experiments in which initiation sites (the sequences where DNA synthesis starts, which are defined by physical methods) were mapped by measuring the time during S phase when a DNA segment replicates or by measuring the average direction of replication fork movement through a DNA segment. In most cases, initiation sites seemed to occur at discrete locations (*8–20*). And, in the three cases where a replicator has been defined (*8, 9, 13, 14*), these initiation sites occur in the same region as the replicator.

However, animal replicators may be less specific or may function differently than those of prokaryotes and budding yeast. For example, in some cases animal cells can initiate replication without replicators or specific initiation sites. DNA molecules injected into *Xenopus* eggs or incubated in egg extracts (*21–23*) replicate once per cell cycle without detectable sequence dependence. Initiation can take place at multiple sites, perhaps randomly, during *Xenopus* embryonic development (*24*), during *Drosophila* embryogenesis (*25*), and in repeated histone genes in cultured (postembryonic) *Drosophila* cells (*26*). In cultured human cells, most genomic sequences that are greater than 10 kilobase pairs can support autonomous plasmid replication (*27*), and replication within these plasmids initiates at multiple, perhaps random, sites (*28*).

The notion that replicators may function differently in animal cells than in yeast and prokaryotes is reinforced by experiments analyzing origins of replication with two-dimensional (2D) gel electrophoretic mapping techniques. These results show that the initiation sites of animal replication origins are distributed over broad regions (initiation zones) of several kilobase pairs or more (*7, 24, 26, 29–32*). When origins have been studied both by 2D gel mapping and by other techniques, the 2D gel results consistently imply that initiation events take place over a broader region than suggested by the other techniques. For the DHFR origin, both replication timing and determination of average fork direction suggest that initiation sites occur within a few kilobase pairs (*8, 10, 13, 14*). However, results from 2D gels suggest that initiation can occur at multiple sites, perhaps anywhere, within an initia-



Events in an animal cell initiation zone. Each pair of parallel blue lines are the parental strands of a DNA molecule in a different cell. The red lines are the newly synthesized daughter strands at initiation sites. [Adapted from (*6*)]

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