MICROBIOLOGY

Malaria's Stealth Shuttle

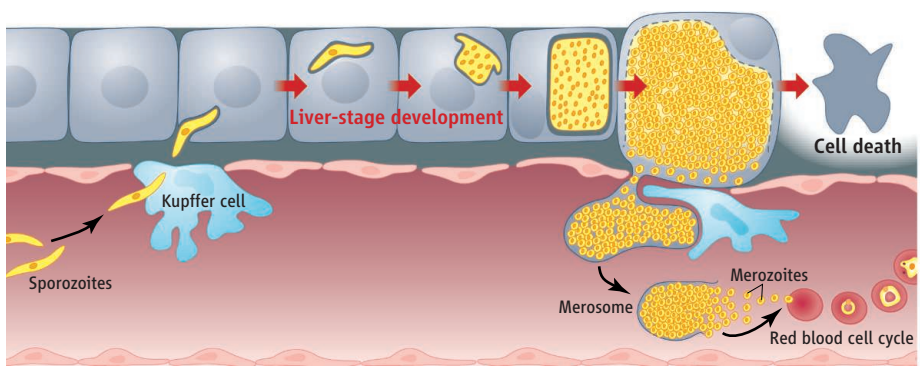
Membranous vesicles shuttle malaria parasites from liver to blood cells during infection, ensuring protection against the host's defenses.

Alan F. Cowman and Stefan H. I. Kappe

In the arms race between pathogens and their hosts, suicide of infected cells is a prominent defense mechanism used by the host to protect against establishment of further infection. Not surprisingly, some pathogens have evolved mechanisms to interfere with host cell death. *Plasmodium* parasites that cause malaria are among the most successful and deadly pathogens of vertebrates, yet the manipulation of molecular pathways responsible for host cell death has only recently emerged as part of their survival toolbox (*1*). On page 1287 of this issue, Sturm *et al.* (*2*) report a major twist to this story during the liver stage of malaria infection. Using *Plasmodium berghei*, a mouse model of malaria, they show that the liver-stage parasite keeps its host hepatocyte alive long enough to complete development but allows it to then commit an unusual form of suicide that helps the parasite evade host defenses and deposit new invasive forms into the bloodstream.

Plasmodium parasites are transmitted to a host organism by the bite of a female *Anopheles* mosquito. The sporozoite form rapidly invades liver hepatocytes (*3*, *4*) and over a number of days, matures as a liver-stage parasite within a membrane-bound vacuolar compartment, shielded from the intracellular milieu of the host cell (see the figure). The liver-stage parasites differentiate into many thousands of merozoites that, when released into the bloodstream, invade erythrocytes, initiating the blood-stage cycle that causes the pathophysiology of malaria.

For liver-stage merozoites to access the bloodstream, they must leave their host cells and cross both an extracellular matrix-filled space and the endothelium of liver blood vessels (sinusoid). Merozoites also must avoid phagocytes in the liver, such as Kupffer cells and dendritic cells, which are eager to attack any foreign invader. Exactly how they overcome these hurdles for release into the bloodstream has been unknown. Using cultured hepatocytes and histological analysis of infected mouse livers, Sturm *et al.* show that late liver-stage parasites dissolve their surrounding vacuolar compartment, thereby gaining access to the hepatocyte cytoplasm. Subsequently, the infected hepatocytes detach from the extracellular substrate and other cells. The authors also identify membrane-enclosed structures—merosomes—that are extruded from the infected cell. In cultured liver cells, merosomes clearly contained merozoites. Real-time intravital imaging of infected mice also revealed merosomes emerging from parasite-infected hepatocytes and entering the sinusoid lumen. The merosomes appear to act as shuttles, allowing safe passage of the parasite into the bloodstream. However, low resolution of the live imaging did not allow the authors to demonstrate unequivocally the presence of fully formed merozoites within merosomes in vivo. Merosome-like structures released into the sinusoids have also been reported recently in the related rodent malaria parasite *Plasmodium yoelii* by intravital imaging (*5*), indicating that these putative shuttles are not a species-specific feature.



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Liver to blood transit. The malaria parasite rapidly grows in liver hepatocytes, producing thousands of merozoites that are competent to infect red blood cells. These liver-stage parasites inhibit hepatocyte cell death during most of their development. When ready to leave hepatocytes, the parasite induces cell death, and causes the release of merozoites in merosomes, thus avoiding host cell defense mechanisms. Merozoites eventually escape from merosomes and infect red blood cells.

What is the benefit to the parasite in packaging liver-stage merozoites into merosomes for delivery to the bloodstream instead of releasing them individually or in one big burst? Sturm *et al.* determined that loss of hepatocyte adhesiveness is associated with host cell death. Although the observed cell death had the hallmarks of classic apoptosis, such as loss of mitochondrial membrane potential and mitochondrial release of cytochrome c, host cell DNA was not characteristically fragmented. Cell death also occurred independent of caspases, proteolytic enzymes whose activity is associated with apoptosis (*6*). However, other cysteine proteases appear to be involved.

Inducing the death of a cell infected with a pathogen seems counterintuitive to providing an advantage for successful infection. In addition to killing the parasite, apoptotic infected cells are also red flags for the immune system. Indeed, *P. berghei* inhibits hepatocyte cell death during most of liver-stage development (*1*). A possible explanation for this twist was found by analyzing the amount of phosphatidylserine in the plasma membrane of infected hepatocytes. In an apoptotic cell, the release of Ca^{2+} from intracellular stores into the cytoplasm causes the amount of phosphatidylserine in the outer leaflet of the plasma membrane to increase substantially. This phosphatidylserine serves as an attraction signal for phagocytic cells (*7*). Sturm *et al.* show that phosphatidylserine is absent in the outer membrane leaflet of dying, infected hepatocytes, thus allowing the hepatocytes to avoid recognition by phagocytic cells. Furthermore, the amount of Ca^{2+} in infected hepatocytes is low, whereas in merozoites it is high, suggesting that the parasite may absorb released Ca^{2+} and thereby suppress exposure of phosphatidylserine on the cell surface. Consistent with this finding, the Ca^{2+} ionophore ionomycin induced phosphatidylserine exposure in infected hepatocytes, and these cells were indeed more frequently phagocytosed compared to untreated infected hepatocytes.

Although the study by Sturm *et al.* provides some answers about the delivery of liver-stage

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merozoites to the bloodstream, there are still many issues to be addressed. How do merosomes, which are large and presumably not motile, pass through the extracellular matrix and sinusoid endothelium? How do merozoites burst out of the merosome once it reaches the bloodstream? How does a liver-stage parasite inhibit the death of the host cell for most of its life but then allow death to occur in a manner that guarantees merozoite progression to blood-stage infection? It is of great interest to determine the molecules and mechanisms that mediate these processes. This is particularly true for *P. falciparum*, which causes the most severe form of malaria and most mortality in humans, because it may reveal potential avenues for the development of novel treatments that block the onset of disease.

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CHEMISTRY

Controlling Biological Functions

Manipulation of the quantum properties of matter can influence the course of biochemical reactions.

Majed Chergui

Can biological functions, such as vision or photosynthesis, that are driven by incoherent phenomena have anything to do with quantum mechanics, where the wave properties of matter play a key role? The answer is yes, and on page 1257 of this issue (*1*), Prokhorenko *et al.* show that biological processes can be manipulated by means of coherent control (*2*).

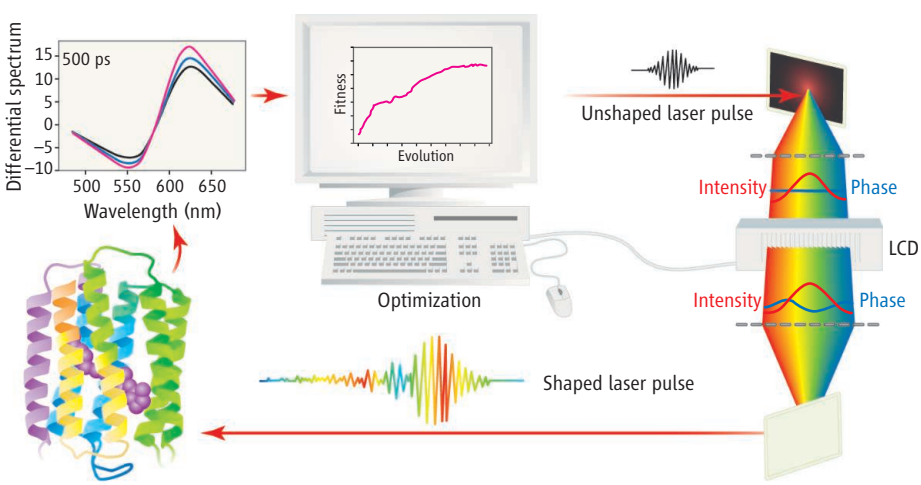
Coherent control refers to experiments that make explicit use of the wavelike nature of matter to direct the behavior of atomic and molecular systems, often to alter the likelihood of a particular chemical reaction. An analogy with Young's double-slit experiment is useful: Light passes through both slits at the same time and interferes with itself at a distant screen to produce dark and bright fringes. To achieve complex interference patterns, however, one needs to control the number, widths, and positions of the slits.

For a quantum mechanical object, one can arrange interference of several "paths" to create constructive interference that selects one state and destructive interference that blocks the others. This is achieved with a pulse of light whose spectral components are controlled in phase and amplitude. This is accomplished by dispersing the different frequency components of the pulse spatially with a diffraction grating, manipulating their phase and amplitude with a spatial mask, and then recombining them to produce a short pulse of well-defined shape. Because adjustment of the relative phases of the components modifies the pulse temporal structure, coherent control can be seen in the time domain as control over a quantum system through manipulation of the temporal structure of the laser field.

The ideal pulse shape is not known a priori, however. In their experiment, Prokhorenko *et al.* used a well-established genetic algorithm inside a feedback loop (*2*) (see the figure). Successful control schemes have previously been demonstrated for specific molecular product channels and states in the gas phase. But most chemical and biological reactions and important physical processes occur in the condensed phase, where interactions of the system with the fluctuating environment may lead to the destruction of the coherence imparted to the system.

Although coherent control has been demonstrated in a variety of condensed-phase systems, including proteins (*3*, *4*), the work of Prokhorenko *et al*. on bacteriorhodopsin contains important novel aspects. First, in all previous coherent control experiments, the shaped laser pulse suppressed unwanted pathways and therefore merely acted as a filter. Examples where the desired target is reached with higher efficiency than with symmetrically shaped pulses are lacking, but Prokhorenko *et al.* were able to increase or decrease the absolute quantum yield of isomerization of the 13-cis retinal isomer in bacteriorhodopsin by as much as 20%, as compared to excitation with symmetric pulses. Second, they used intensities of the exciting light comparable to that of sunshine, relevant to photobiological processes. This is important for controlling the actual reaction coordinate rather than the excited-state population (*4*), and in interpreting the correlation of the shaped pulses to the molecular processes. And third, at the end of the optimization (or antioptimization) control loop, their pulse shapes capture the underlying molecular dynamics driving the process, and show a selective coherent excitation of precisely those torsional modes responsible for isomerization.

But why perform coherent control in bio-



In the loop. A feedback loop is used to control isomerization of bacteriorhodopsin. An initial laser control field is created with a pulse shaper (on the right) and is then applied to the protein sample. The action of the control pulse, measured as the difference spectrum of the sample, is used by a learning algorithm to produce an improved field. Repeated excursions around the loop result in an optimum control.

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