# Arsenic(III) Fuels Anoxygenic Photosynthesis in Hot Spring Biofilms from Mono Lake, California

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overexpressing HopT1-1 (fig. S10) exhibited attenuated chlorosis (Fig. 3E) and accumulated higher *SUL* mRNA levels (Fig. 3G). However, the *SUL* siRNA levels remained unchanged (Fig. 3D), mimicking the reported effects of the *ago1-12* mutation in SS (*23*). Also as in *ago1-12*, canonical miRNAs accumulated normally in HopT1-1–overexpressing lines, despite higher levels of miRNA target transcripts (Fig. 3, F and G, and fig. S11), suggesting that HopT1-1 probably suppresses slicing mediated by AGO1. Further transient overexpression of HopT1-1 in *efr* plants showed a dramatic increase in the protein, but not mRNA, levels of the miR834 target COP1-interacting protein 4 (CIP4) (Fig. 3H and fig. S12, A and B). Thus, HopT1-1 additionally, and perhaps predominantly, suppresses miRNA-directed translational inhibition, which is consistent with the involvement of AGO1 in this second process (*24*). Similarly, higher protein levels of CIP4 and of the copper/zinc superoxide dismutase 1 (CSD1-miR398 target) were detected in plants infected with virulent *Pto* DC3000 (Fig. 3H and fig. S12C), with no effect on CSD1, CIP4, and some other miRNA target transcript levels (fig. S13).

We show here that the miRNA pathway plays a major role in antibacterial basal defense and, accordingly, we have identified bacterial suppressors of RNA silencing, or BSRs. This finding provides a plausible explanation for the synergistic interactions observed in the field between some viral and bacterial phytopathogens. Consistent with this idea, we found that infection by *Turnip Mosaic Virus* (TuMV), which produces the P1-HcPro suppressor of siRNA and miRNA functions (*25*, *26*), reduces basal and nonhost resistances to promote growth and diseaselike symptoms from nonvirulent *Pto* DC3000 *hrcC*$^-$ and *Psp* NPS3121 bacteria (Fig. 4). It will now be important to elucidate how silencing factors are modified by VSRs and BSRs, and whether such modifications are sensed by specific resistance (R) proteins as postulated by the "guard hypothesis" (*27*).

The implication of the miRNA pathway in innate immunity is not specific to plants. For example, human *MiR146* is induced by several microbial components (*28*). Because type III secretion systems are widespread across Gram-negative bacteria (*29*), the intriguing possibility emerges that human pathogenic bacteria also have evolved to suppress RNA silencing to cause disease.

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### Supporting Online Material

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# Arsenic(III) Fuels Anoxygenic Photosynthesis in Hot Spring Biofilms from Mono Lake, California

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Phylogenetic analysis indicates that microbial arsenic metabolism is ancient and probably extends back to the primordial Earth. In microbial biofilms growing on the rock surfaces of anoxic brine pools fed by hot springs containing arsenite and sulfide at high concentrations, we discovered light-dependent oxidation of arsenite [As(III)] to arsenate [As(V)] occurring under anoxic conditions. The communities were composed primarily of *Ectothiorhodospira*-like purple bacteria or *Oscillatoria*-like cyanobacteria. A pure culture of a photosynthetic bacterium grew as a photoautotroph when As(III) was used as the sole photosynthetic electron donor. The strain contained genes encoding a putative As(V) reductase but no detectable homologs of the As(III) oxidase genes of aerobic chemolithotrophs, suggesting a reverse functionality for the reductase. Production of As(V) by anoxygenic photosynthesis probably opened niches for primordial Earth's first As(V)-respiring prokaryotes.

Anoxygenic photosynthesis, common to photosynthetic bacteria and certain cyanobacteria (e.g., *Oscillatoria*), harvests electrons from low–electrochemical potential donors and shunts them toward the reduction of $CO_2$ for incorporation into biomass. This mechanism of photoautotrophy typically uses hydrogen sulfide as an electron donor and a single photosystem as a catalyst, oxidizing sulfide to sulfur and sulfate to support the growth of phototrophs in anoxic sulfidic environments exposed to light, such as laminated microbial mats (*1*, *2*) and the pycnoclines of stratified lakes (*3*, *4*).

Oxygenic photosynthesis characteristic of plants, algae, and cyanobacteria uses two photosystems and harvests electrons from water, resulting in the evolution of molecular oxygen. Oxygenic photosynthesis changed Earth's atmosphere from reducing to oxidizing, a process that could have begun as early as 2.7 billion years ago (Ga) during the late Archean (3.8 to 2.5 Ga) based on the presence of cyanobacterial biomarkers in sedimentary rocks of this age (*5*). However, the key cyanobacterial biomarkers in ancient rocks, namely 2-methyl hopanes (*6*), also occur (as 2-methylhopane polyols) in photosynthetic bacteria (*7*); both cyanobacteria and photosynthetic bacteria appear to have formed the laminated mat structures found in ancient sedimentary rocks (*8*). Hence, anoxygenic photosynthesis may well have been widespread in the Archean (*9*). This conclusion is underscored by the discoveries that in addition to reduced sulfur compounds, $Fe^{2+}$ (*10*, *11*) as well as $NO_2^-$ (*12*) serve as electron donors for anoxygenic photosynthesis carried out by photosynthetic bacteria.

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Here, we supply evidence for As(III)-supported anoxygenic photosynthesis in naturally occurring microbial mats of cyanobacteria and photosynthetic bacteria from Mono Lake, California, hot springs and provide evidence of photoautotrophic growth of pure cultures of purple bacteria supported by As(III) oxidation to As(V). Because the only previously reported biogenic oxidation of As(III) was by chemolithotrophic (nonphototrophic) bacteria that require strong oxidants such as oxygen (*13*) or nitrate (*14*), it was assumed that dissimilatory arsenic reduction, a widely distributed microbial process that requires As(V) as an electron acceptor, arose only after the evolution of oxygenic photosynthesis (*15*). The ability of phototrophic prokaryotes to generate As(V) from As(III) under anoxic conditions (electrochemical potential = $E_o'$ $AsO_3^{3-}/AsO_4^{3-}$ = +139 mV) requires that the antiquity of prokaryotic arsenate respiration be reevaluated and has important implications for all aspects of the arsenic cycle.

We sampled two small hot spring–fed ponds, termed "green" and "red," located on the southeastern shore of Paoha Island in Mono Lake, California (37°59.633′ N, 119°01.376′ W), an area with numerous gaseous hydrocarbon seeps (*16*). Both habitats were alkaline, anoxic, and saline, and both contained arsenite and arsenate at high concentrations, as well as other dissolved reductants (methane, sulfide, and ammonia). The hot springs and ponds of Paoha Island lacked any visual evidence of Fe(III) oxides typical of acidic or circumneutral hot springs and also had low dissolved Fe(II) concentrations (≤0.7 μM). Although of similar chemistry (Table 1), the two ponds showed pronounced color differences due to the 1- to 2-mm-thick biofilms that completely covered their submerged rock cobble surfaces (Fig. 1). The green biofilm (67°C, Fig. 1A) was dominated by filamentous *Oscillatoria*-like cyanobacteria (Fig. 1B), whereas the red biofilm (43°C, Fig. 1C) contained mainly purple photosynthetic bacteria (Fig. 1 D). Obtaining smooth absorption spectra of the original mat material was complicated by the abundance of inorganic material present in the samples. However, small peaks at 850 and 795 nm, suggestive of bacteriochlorophyll a, were obtained from the red mat sample, and peaks at 664 and 638 nm, suggestive of chlorophyll a and phycocyanin, respectively, were obtained from the green mat sample.

Incubation of slurried biofilms showed As(III) oxidation linked to anoxygenic photosynthesis (*17*). Slurries from the green mat slowly oxidized As(III) to As(V) under anoxic conditions in the light but not in the dark (Fig. 2A) or in heat-killed controls incubated in the light (fig. S1A). Similarly, red mat material also oxidized As(III), and subsequent injected pulses of 1 mM and 5 mM As(III) were completely oxidized to As(V) (Fig. 2B). In contrast, a 0.25 mM As(III) addition was not oxidized by dark, anoxic controls or by killed controls incubated in the light (fig. S1, B and C). Red mat material incubated in an $N_2$ atmosphere oxidized ~1 mM As(III) completely to As(V) within 20 hours, with no corresponding oxidation occurring in the dark-incubated controls under $N_2$ (Fig. 2C). Similarly, oxidative loss of sulfide occurred under light/anoxic but not dark/anoxic conditions (Fig. 2D). Red mat material also demonstrated incorporation of $^{14}C$-bicarbonate into cell material when incubated in the light under anoxic conditions with the following electron donors (percent radiotracer incorporated ± 1 SD, $n$ = 3 slurries): none (0.29 ± 0.02), 100% methane headspace (0.28 ± 0.03), 2 mM As(III) (0.48 ± 0.04), and 2 mM sulfide (1.47 ± 0.23). In contrast, anoxic dark-incubated mat samples that were otherwise incubated as above all incorporated ≤0.007% (SD ≤ 0.002) of the added tracer. These results demonstrate that photoautotrophic $CO_2$ fixation can be linked to As(III) oxidation by cells in the red biofilm.

To obtain conclusive evidence for As(III)-linked anoxygenic photosynthesis, we isolated two axenic strains of a photosynthetic purple bacterium that displayed As(III)-dependent growth (*17*). One isolate, designated strain PHS-1 and studied in detail, oxidized As(III) and grew as a photoautotroph reaching high cell densities (Fig. 3, A and B). In contrast, strain PHS-1 did not grow in light-incubated control cultures lacking As(III), or in dark-incubated cultures that contained As(III) (Fig. 3B), in which no production of As(V) occurred (i.e., control arsenate concentrations remained at the carry-over levels of ~0.9 ± 0.1 mM throughout the 200 hours of incubation). Arsenite additions (2 or 4 mM) were made as a series of injections over the time course because high initial arsenite concentrations (~5 mM) were found to inhibit growth. In such experiments, a cumulative total of 12 mM injected As(III) was stoichiometrically converted to As(V) in phototrophic cultures (Fig. 3A). Growing cell suspensions of strain PHS-1 turned from light pink to the deep red color of natural populations (Fig. 1C), and absorption spectra confirmed that the cells contained bacteriochlorophyll a. Peaks near 800, 850, and 880 nm in the spectrum of intact cells of strain PHS-1, along with the major peak at 769 nm in methanol extracts of cells, are typical of purple bacteria containing bacteriochlorophyll a (fig. S2).

Cells of strain PHS-1 grown phototrophically on As(III) were motile and indistinguishable from

**Table 1.** Physical and chemical properties of the two Paoha Island hot spring–fed ponds compared with Mono Lake surface water.

| Parameter | Red pond | Green pond | Mono Lake* |
|---|---|---|---|
| Temperature (°C) | 43 | 67 | 20 |
| Salinity (g/liter) | 25 | 32 | 84 |
| Dissolved $O_2$ (μM) | 1.0 | 1.6 | 120 |
| pH | 9.4 | 9.3 | 9.8 |
| Arsenite (μM) | 92.0 | 68.4 | 0 |
| Arsenate (μM) | 9.7 | 65.1 | 200 |
| Sulfide (mM) | 5.8 | 5.0 | 0 |
| Ammonia (mM) | 1.2 | 1.4 | 0 |
| Dissolved organic carbon (mM) | 2.2 | ND† | 6.7 |
| Methane (μM) | 430 | 360 | 1.0 |
| Ethane (μM) | 4.0 | 4.0 | 0 |

*Mono Lake surface water data compiled from several sources (*17*). †ND, not determined.

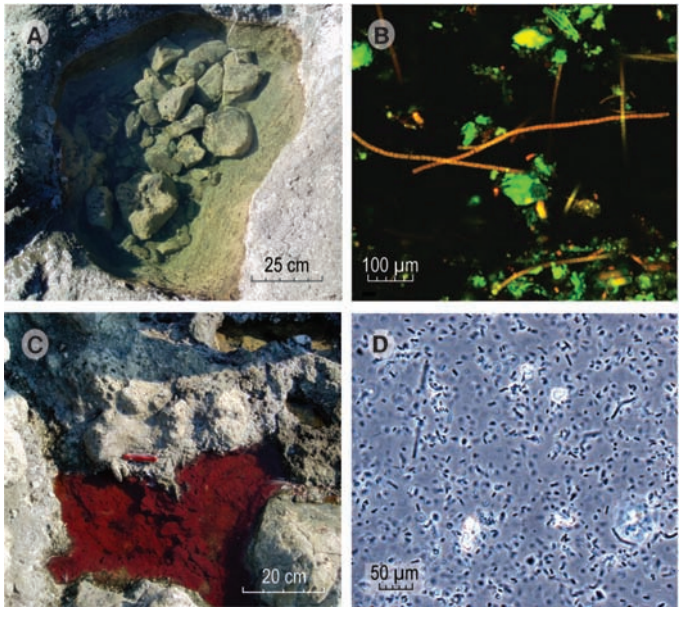


**Fig. 1.** Photographic and microscopic images of the hot spring–fed pools on Paoha Island. (**A**) Green pool. (**B**) Confocal microscopic image of green pool biofilm microbes showing a fluorescing *Oscillatoria*-like cyanobacterium. (**C**) Red pool. (**D**) Photomicrographic image of red pool biofilm material composed primarily of *Ectothiorhodospira*-like bacteria.

sulfide-grown cells (fig. S3). Phylogenetic analysis of 16*S* ribosomal RNA (rRNA) gene sequences indicated that strain PHS-1 is a member of the gamma-Proteobacteria genus *Ectothiorhodospira* and most closely related to the species *Ectothiorhodospira shaposhnikovii* (99.4% 16*S* rRNA gene sequence identity, Fig. 3C). The 16*S* rRNA gene sequence of strain PHS-1 was identical to that of strain MLP2, a second organism obtained from the red mat, but isolated with sulfide instead of As(III). Photoautotrophic growth of strain MLP2 was also stimulated by As(III), because growth yields in media containing 1 mM each of sulfide and As(III) were ~50% higher than controls containing sulfide alone. This suggests that the electron donor (sulfide versus arsenite) used for enrichment and isolation was not a factor in selecting for the As(III)-oxidizing phenotype. However, *Ectothiorhodospira* sp. strain ML Ecto, a purple bacterium previously isolated from an As(III)-oxidizing photosynthetic enrichment culture obtained from Mono Lake sediment (*18*), did not grow photoautotrophically on As(III).

Our experimental observations of the green mats (Fig. 2A) suggested that the *Oscillatoria*-type cyanobacteria that dominated the mat may also be capable of As(III)-supported anoxygenic photosynthesis. The pattern of light-dependent As(III) oxidation by the cyanobacterial mat was very similar to that of the red mat (Fig. 2, B and C), although the results with the green mat have not yet been confirmed with pure cultures in the

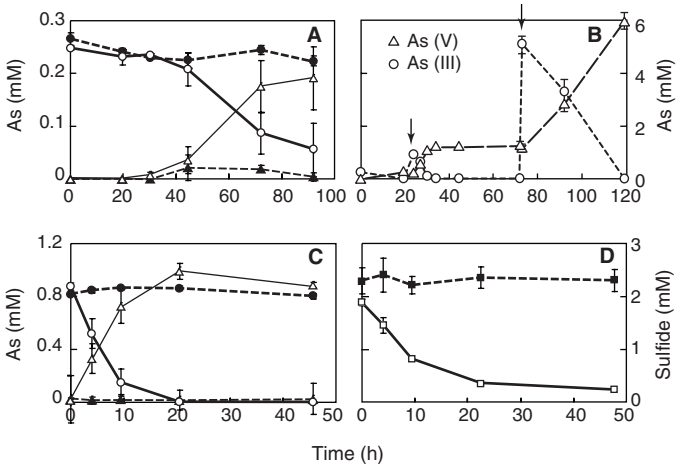


**Fig. 2.** Time-course incubations of slurried biofilm material incubated under anoxic conditions in the light (open symbols) or dark (closed symbols) at 45°C. Circles represent As(III), triangles represent As(V), and squares represent sulfide. Results represent the mean of three slurries, and bars indicate ±1 SD. (**A**) Oxidation of As(III) to As(V) by green pool biofilms. (**B**) Oxidation of As(III) to As(V) by red pool biofilms. Arrows indicate additions of As(III). (**C**) Short-term oxidation of As(III) to As(V) in light-incubated and dark-incubated red pool biofilms. (**D**) Oxidative loss of sulfide by red pool biofilms incubated in the light or dark.

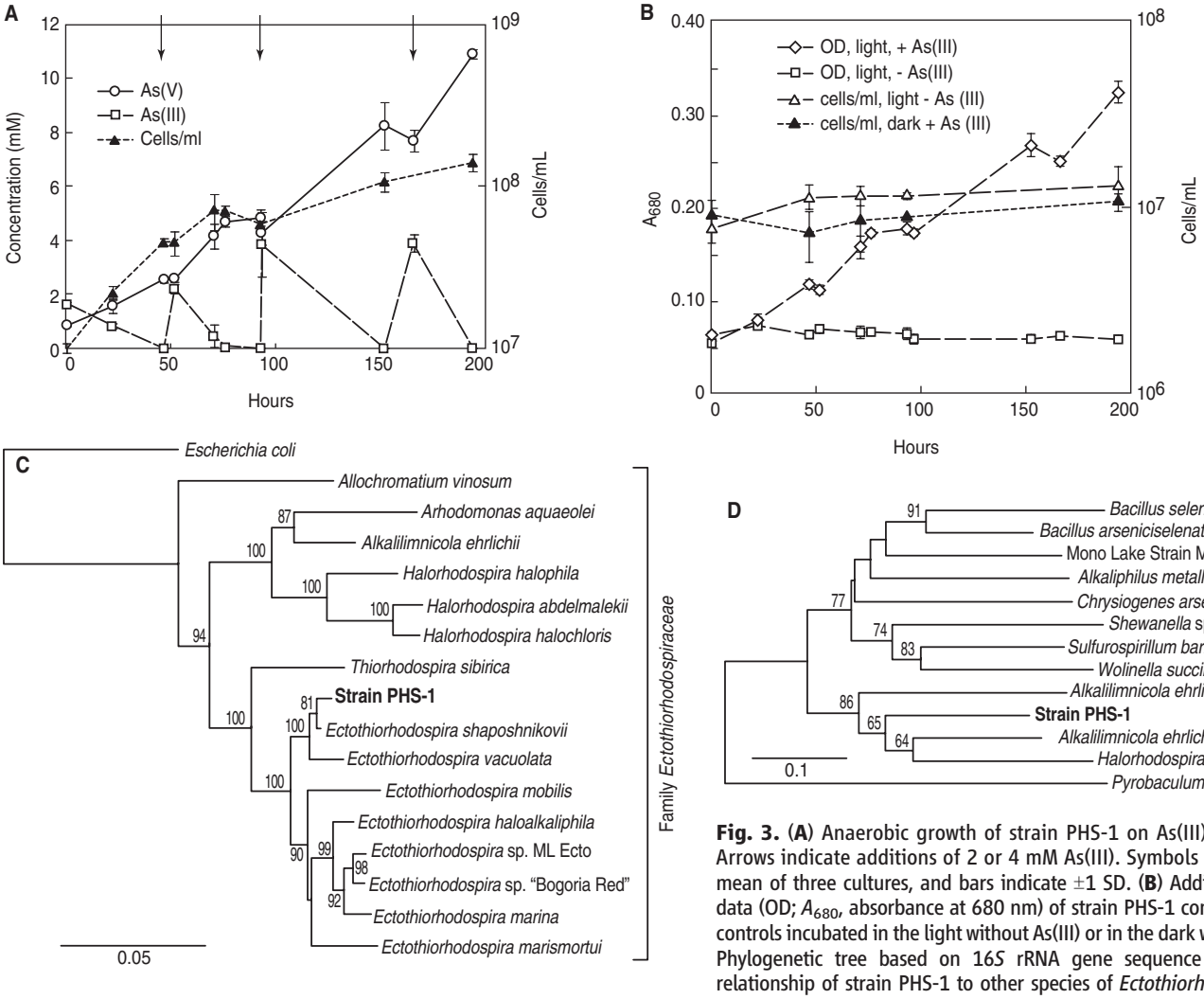


**Fig. 3.** (**A**) Anaerobic growth of strain PHS-1 on As(III) in the light. Arrows indicate additions of 2 or 4 mM As(III). Symbols represent the mean of three cultures, and bars indicate ±1 SD. (**B**) Additional growth data (OD; $A_{680}$, absorbance at 680 nm) of strain PHS-1 compared to live controls incubated in the light without As(III) or in the dark with As(III). (**C**) Phylogenetic tree based on 16*S* rRNA gene sequence showing the relationship of strain PHS-1 to other species of *Ectothiorhodospiraceae*. All sequences have been deposited in GenBank, and accession numbers are listed in the supporting online material (SOM). (**D**) Phylogenetic tree based on ArrA-like gene sequences showing the relationship between strain PHS-1 genes with others from the GenBank database. Accession numbers are given in the SOM.

laboratory as was done with strain PHS-1 (Fig. 3, A and B). Nevertheless, pure cultures of certain cyanobacteria (e.g., *Oscillatoria limnetica*) are known to use sulfide as an electron donor in anoxygenic photosynthesis (*19*). The oxidation of As(III) to As(V) by the Paoha cyanobacteria may be a second example of a two-electron transition—being analogous to the oxidation of $H_2S$ to $S^0$ by *O. limnetica* (*19*). Phototrophic microorganisms may also contribute to As(III) oxidation in other environments, such as the chemically diverse hot springs of Yellowstone National Park, many of which contain arsenite (*20*–*22*), thereby broadening the ecological importance of the phenomenon described here.

Despite the clear evidence for arsenite oxidation by strain PHS-1, we were unable to obtain an amplicon for arsenite oxidase (*aoxB*) (*17*). This result was surprising because the gene is highly conserved across broad phylogenetic lineages (*23*) and primer sets have been successfully used for identifying *aoxB* in both pure cultures and environmental samples (*22*, *24*). An amplicon was obtained when primers for dissimilatory arsenate reductase designed for halophilic prokaryotes (*25*) were used. This occurred even though strain PHS-1 did not grow in the dark with 10 mM As(V) as the electron acceptor and 10 mM lactate, 10 mM acetate, or 4 mM sulfide as the electron donor [i.e., no loss of As(V) or production of As(III) and all optical densities (ODs) remained below 0.07 after 8 days of incubation]. The putative ArrA homolog for strain PHS-1 showed a high degree of sequence identity (~68%) to proteins from two other *Ectothiorhodospiraceae*, *Alkalilimnicola ehrlichii* and *Halorhodospira halophila* (Fig. 3D). *A. ehrlichii* is a nonphototrophic As(III)-oxidizing chemolithoautotroph that was isolated from the water column of Mono Lake (*14*). Analysis of its entire annotated genome revealed that it also lacks genes encoding an arsenite oxidase (e.g., *aoxAB*) but it does have two putative *arr* operons (*14*). Arsenic metabolism was not studied in *H. halophila* (*26*) although its genome contains a homolog of *arrA* (Fig. 3D) annotated as a "formate dehydrogenase." These results suggest that in the *Ectothiorhodospiraceae*, the Arr homolog is functioning in reverse or that an unknown mechanism for arsenite oxidation exists that carries out this process under anoxic conditions. Either way, it appears there are at least two distinct mechanisms for arsenite oxidation.

Over the past decade, several phylogenetically diverse microorganisms have been described that conserve energy from the oxidation or reduction of arsenic oxyanions and include deep lineages of both Bacteria and Archaea (*15*). In certain environments, a robust arsenic biogeochemical cycle supports a diverse microbial community (*25*, *27*). Our discovery of anaerobic photosynthetic oxidation of As(III) adds an important new dimension to the arsenic cycle and highlights a previously unsuspected mechanism that may have been essential for establishing and maintaining the arsenic cycle on the ancient anoxic Earth.

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### Supporting Online Material

www.sciencemag.org/cgi/content/full/321/5891/967/DC1
Methods
Figs. S1 to S3
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# In Vivo Imaging Reveals an Essential Role for Neutrophils in Leishmaniasis Transmitted by Sand Flies

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**Infection with the obligate intracellular protozoan *Leishmania* is thought to be initiated by direct parasitization of macrophages, but the early events following transmission to the skin by vector sand flies have been difficult to examine directly. Using dynamic intravital microscopy and flow cytometry, we observed a rapid and sustained neutrophilic infiltrate at localized sand fly bite sites. Invading neutrophils efficiently captured *Leishmania major* (*L.m.*) parasites early after sand fly transmission or needle inoculation, but phagocytosed *L.m.* remained viable and infected neutrophils efficiently initiated infection. Furthermore, neutrophil depletion reduced, rather than enhanced, the ability of parasites to establish productive infections. Thus, *L.m.* appears to have evolved to both evade and exploit the innate host response to sand fly bite in order to establish and promote disease.**

Many parasitic diseases are transmitted by the bite of an infected arthropod, yet the dynamics of the host-parasite interaction in this context remain largely uncharacterized. Transmission of *Leishmania* by infected sand fly bite represents an attractive experimental system to study early inflammatory responses and relate these processes to the establishment of an infectious disease. Leishmaniasis is thought to be initiated by direct parasitization of macrophages after deposition into the skin (*1*). However, the ability of neutrophils to rapidly respond to and efficiently phagocytose a variety of pathogens suggests that they may also be an initial target of *Leishmania* infection (*2*–*4*). Indeed, after needle injection of *Leishmania major* (*L.m.*), infected neutrophils have been observed, and both host-protective and disease-promoting roles for these cells have been reported (*5*–*10*). How-

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