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17. To sequence the mutations in *n821*, *n1168*, and *n1169*, we amplified genomic DNA fragments from the *let-2* gene in the three mutants by polymerase chain reactions, using KlenTaq LA polymerase [W. M. Barnes, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 2216 (1994)]; their sequences were then determined. To confirm the mutation identified by DNA sequencing, we used a 797-bp Bst XI–Nde I fragment (which spanned the mutation from the mutant DNA) to replace the same region in the wild-type clone and introduced the resulting plasmid into an *unc-105(n490); let-2(n821)* strain. Transformed animals identified through the Roller phenotype induced by the pRF4 carrier DNA were otherwise normal in motility, which indicates that the plasmid contains the suppressor allele. A similar result was obtained when a rescuing line was first established in a *let-2(g30ts)* background and then crossed into *unc-105(n490); let-2(n821)*.
18. It seems unlikely that the suppression is caused by general decreases in the collagen function, as we did not observe suppression of *unc-105(n490)* by *let-2(g30ts)* even at intermediate temperatures.
19. We constructed an *unc-105::gfp* promoter fusion by fusing the 5.1-kb genomic fragment upstream of the start codon of *unc-105* with the *gfp* expression vector pPD95.73 (A. Fire, J. Ahnn, G. Seydoux, S. Xu, personal communication). When this construct was introduced into wild-type N2 animals, we observed nuclear-localized fluorescent signals in body-wall muscle cells and did not detect expression in hypodermal or motor neuron cells.
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30. We thank R. Horvitz for *unc-105* and *sup-20* strains; J. Kramer for *let-2* plasmids; A. Fire for *gfp* vector plasmids; M. Driscoll, G. Kao, J. Kramer, and W. Wadsworth, for sharing unpublished data; and C. Colledge, M. Hresko, P. Hoppe, M. Johnston, M. Nonet, T. Schedl, J. Waddle, and B. Williams for comments on the manuscript and helpful discussions. Some nematode strains used in this work were provided by the *Caenorhabditis* Genetics Center, which is funded by the NIH National Center for Research Resources (NCRR). This work was supported by NIH grant GM23883 to R.H.W and by Muscular Dystrophy Association fellowships to J.-D.L. and B.S.

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Oxygenic Photoautotrophic Growth Without Photosystem I

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Contrary to the prediction of the Z-scheme model of photosynthesis, experiments demonstrated that mutants of *Chlamydomonas* containing photosystem II (PSII) but lacking photosystem I (PSI) can grow photoautotrophically with O_2 evolution, using atmospheric CO_2 as the sole carbon source. Autotrophic photosynthesis by PSI-deficient mutants was stable both under anaerobic conditions and in air (21 percent O_2) at an actinic intensity of 200 microeinsteins per square meter per second. This PSII photosynthesis, which was sufficient to support cell development and mobility, may also occur in wild-type green algae and higher plants. The mutants can survive under 2000 microeinsteins per square meter per second with air, although they have less resistance to photoinhibition.

In the Z scheme, first proposed by Hill and Bendall (*1*), PSII can split water but is not thought to be able to perform one of PSI's assigned functions: the reduction of ferredoxin (Fd)/nicotinamide adenine dinucleotide phosphate ($NADP^+$), which is essential for CO_2 assimilation. The Z scheme therefore requires that both PSII and PSI work in sequence for complete photosynthesis, using water as the source of electrons and CO_2 as the terminal electron acceptor. Despite some disagreement (*2–5*), the Z scheme has become the textbook model of photosynthesis (*6*, *7*). Sustained photoassimilation of CO_2 and evolution of H_2 and O_2 in minimal medium can be achieved by the PSII light reaction without involvement of PSI in a PSI-deficient mutant of *Chlamydomonas* grown photoheterotrophically with the use of an organic nutrient (acetate) (*8*). Here we report that PSI-deficient mutants of *Chlamydomonas* were capable of growing photoautotrophically. Because the Z scheme requires both PSI and PSII working in series, it predicts that PSI-deficient mutants of green algae will not grow photoautotrophically. The discovery of photoautotrophic growth of PSI-deficient green algae without any organic nutrients, therefore, suggests the existence of PSII photosynthesis that is an alternative to the Z scheme. Our discovery may provide an explanation for many reports of anomalous quantum requirements that cannot be explained by the Z scheme.

Photoautotrophic growth of several PSI-deficient mutants of *Chlamydomonas*, such as F8 and B4, was observed when photoheterotrophically grown aliquots were inoculated into 75 ml of minimal medium (*9*) in sterilized Erlenmeyer flasks and incubated under continuous actinic illumination [photosynthetically active radiation (PAR) of 20 microeinsteins (μE) m^{-2} s^{-1}] provided by daylight fluorescent lamps (an einstein is the energy of 1 mol of photons). The minimal medium contained only water and mineral elements. The flasks that contained the liquid culture were capped with phenolic screw caps that allowed air (CO_2) exchange. Constant shaking of the culture flasks with a gyratory shaker at a speed of 140 rpm facilitated air exchange for CO_2 supply. Under these conditions, the algal cultures grew for

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months. The initial population immediately after inoculation was about 5.0×10^4 cells per milliliter for all cultures. Based on population growth, monitored by counting of algal cell density in the media with a microscopic hemacytometer, the algal cell doubling time in their logarithmic growth phase was 10, 10, and 6.7 days for F8, B4, and wild-type 137c, respectively, at the relatively low light intensity of 20 $\mu E\ m^{-2}\ s^{-1}$. The logarithmic phase lasted about 40 to 60 days, followed by a nearly linear growth phase. After about 100 days of incubation, the cell populations were more than 50 times the size of the initial population.

With the use of the above photoautotrophically grown cultures as inocula, photoautotrophic growth was further demonstrated at moderate actinic intensity (PAR, 200 $\mu E\ m^{-2}\ s^{-1}$), where the growth was much faster. In these experiments we studied photoautotrophic growth in vertical glass growth tubes that were specially designed for algal growth and simultaneous measurement of CO_2 photoassimilation and O_2 and H_2 evolution, using an in-line CO_2 analyzer (LI-6252, Li-Cor Instruments) with a flow-detection system described previously (*10*). Day and night cycles were mimicked by 12 hours of darkness and 12 hours of illumination (200 $\mu E\ m^{-2}\ s^{-1}$), provided by daylight fluorescent lamps that were controlled by an electronic timer. The liquid culture in each tube (38 cm long and 3 cm in diameter) was continuously bubbled with 700 parts per million (ppm) CO_2 in helium or air at a rate of 50 ml min^{-1} to supply CO_2 and to agitate the algal suspension. Algal growth was measured by cell population and chlorophyll (chl) content. Both chl content and cell population rose quickly with time (Fig. 1). With the supply of 700 ppm CO_2 in air and 200 $\mu E\ m^{-2}\ s^{-1}$ of illumination, the chl doubling times were 1.0, 1.1, and 0.60 days for F8, B4, and 137c, respectively, which lasted for 2 to 3 days. Initial exponential growth, followed by linear growth as self-shading and nutrient depletion set in, is the normal pattern for photoautotrophically grown algae.

The mutants were able to grow not only in air but also in anaerobiosis when CO_2 was supplied in a pure helium atmosphere (Fig. 1). The pattern of photoautotrophic growth under CO_2 in helium was similar to that for CO_2 in air. This indicates that neither aerobic processes such as mitochondrial respiration or chlororespiration (*11*, *12*) nor anaerobic hydrogenase activity (*13*) is required for cell growth. Although the absence of atmospheric O_2 slowed the growth of the wild type by about 7%, it did not affect the

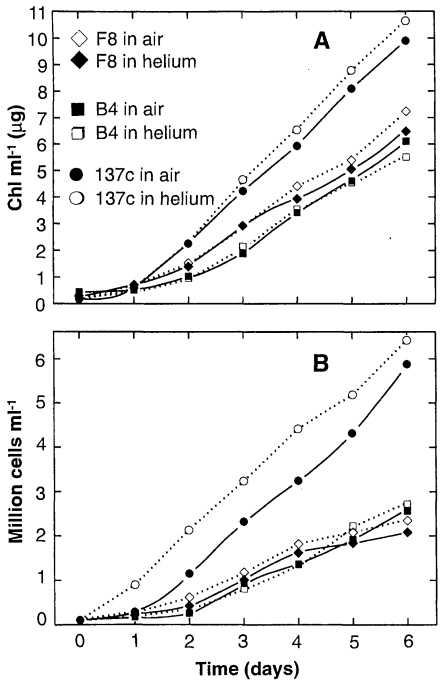

Fig. 1. Photoautotrophic growth curves of PSI-deficient mutants F8 (diamonds) and B4 (squares) and wild-type 137c (circles) in the presence of 700 ppm CO_2 in either air (open symbols) or helium (solid symbols) during cycles of 12 hours of darkness and 12 hours of light (PAR, 200 $\mu E\ m^{-2}\ s^{-1}$). The population growth was measured by chl content (**A**) and cell density (**B**) of the cultures in the minimal medium. The preculture was prepared on a rotary shaker under overhead room lights; atmospheric CO_2 was the sole carbon source. The growth experiment used a specially designed flow system with CO_2 in the carrier gas and a bank of fluorescence lamps for illumination.

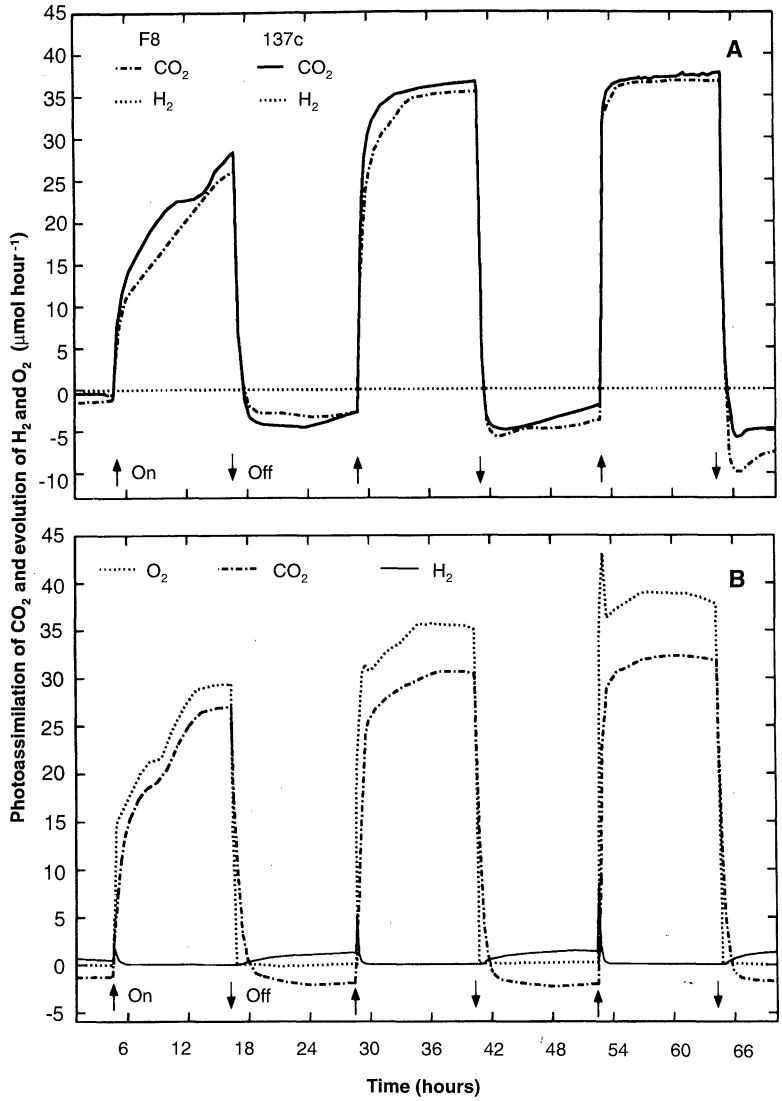

Fig. 2. (**A**) Photoassimilation of CO_2 and zero-rate production of H_2 by mutant F8 and wild-type 137c in the presence of 700 ppm CO_2 in air. (**B**) Photoassimilation of CO_2 and evolution of O_2 and H_2 by F8 in the presence of 700 ppm CO_2 in helium. In each case, the initial algal population in the minimal medium (140 ml) was such that the chl concentration was about 0.4 $\mu g\ ml^{-1}$. The actinic illumination (PAR, 200 $\mu E\ m^{-2}\ s^{-1}$) was provided by daylight fluorescent lamps. The upward- and downward-pointing arrows mark the on and off phases, respectively, of the actinic illumination.

growth rate for F8 and B4, which was supported solely by PSII photosynthesis.

The evidence that the carbon and electrons needed for cell growth are acquired from CO_2 fixation and water splitting was obtained by simultaneous measurement of CO_2, O_2, and H_2 in the carrier gas effluent after bubbling through the growth tubes described above. Both CO_2 uptake and O_2 evolution were light dependent (Fig. 2). Photoassimilation of CO_2 by the PSI-deficient mutants was stable in both aerobic (Fig. 2A) and anaerobic conditions (Fig. 2B). During steady-state photosynthesis, about 40% of the CO_2 supplied by the carrier gas was taken up by the algae, corresponding to a maximal photosynthesis rate of about 100 μmol of CO_2 $hour^{-1}$ per milligram of chl. Under aerobic conditions, hydrogenase (H_2 production) was inactivated (Fig. 2A). However, the pattern and rates of CO_2 photoassimilation under aerobic conditions were similar to those under anaerobic conditions (Fig. 2B). These results indicated that neither hydrogenase activity nor any respiratory process (such as mitochondrial respiration or chlororespiration) is required by PSII photosynthesis. Even under anaerobic conditions in which hydrogenase is activated, photoevolution of H_2 occurred only upon the initial onset of actinic illumination when the Calvin cycle for CO_2 reduction had not been fully activated. At steady state, H_2 evolution approached zero and reduction of CO_2 by the Calvin cycle became the exclusive sink for reductant generated by photosynthetic water splitting.

The simultaneous measurements of CO_2 uptake and O_2 evolution (Fig. 2B) also revealed that the photosynthetic quotient (CO_2 uptake/O_2 evolved) can be less than unity. After the first light cycle, the quotient (CO_2/O_2) during steady-state photosynthesis was as low as 0.90 in both the PSI-deficient algae and the wild type. The observed photosynthetic quotient is expected if CO_2 is reduced not only to the level of sugars, which would correspond to a quotient of unity, but also to the level of lipids and proteins, both of which are necessary for cell growth and correspond to photosynthetic quotients of <1 (*14*). The measured photosynthesis therefore is consistent with the observed cell growth. PSII photosynthesis also supports cell development.

At the early stage of culture, within 30 days of illumination at 20 μE m^{-2} s^{-1} (or within the first 2 days at 200 μE m^{-2} s^{-1}), the PSI-deficient algae were mostly static green cells lacking flagella. The cellular volume, however, was about three to four times larger than that of the wild-type cells. After about 60 days of incubation under 20 μE m^{-2} s^{-1} (or after 3 days under 200 μE m^{-2} s^{-1}), many of the mutant cells developed flagella and swam actively. Similar development was observed under anaerobic conditions.

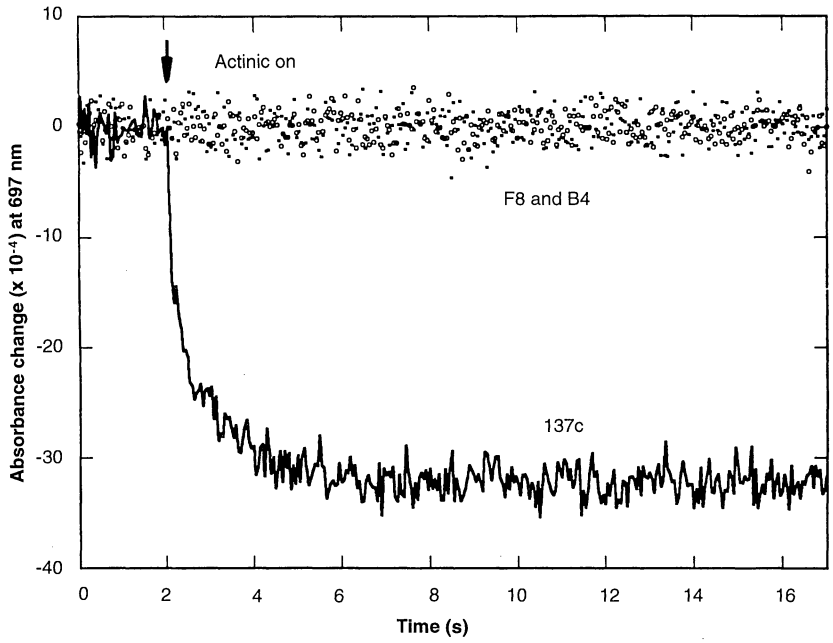

Fig. 3. A typical set of reversible P700 photo oxidation data obtained from thylakoids that were isolated from photoautotrophically grown cells of mutants F8 (solid symbols) and B4 (open symbols) and wild-type 137c (line). The thylakoids were treated with cyanide (CN^-) to inhibit plastocyanin, which otherwise may interfere with P700 measurement by donating its electrons to the PSI reaction centers. The chl concentration was 47 μM for all samples (thylakoids). Before the measurements, the samples were dark-adapted in the presence of ascorbate so that the PSI reaction centers were all kept in their reduced state (P700). The photoconversion of P700 to $P700^+$ was measured as the absorbance change at 697 nm. The downward-pointing arrow marks the onset of actinic illumination.

Mutant F8 was obtained from mutagenesis by ultraviolet treatment and was characterized as a stringent mutant that completely lacks the core of PSI (CPI) and two low molecular weight (20 and 21 kD) polypeptides (*15*). Mutant B4 was obtained after metronidazole enrichment (*16*). Before and after our growth experiments, the PSI content of these mutant algae was examined and the complete absence of PSI was confirmed. PSI reaction center (P700) photochemical activity was measured by absorbance spectroscopy in thylakoid membranes that were isolated from these photoautotrophically grown algal cells (Fig. 3). No P700 activity was detected in either F8 or B4 mutants, whereas the P700 signal was easily seen in the wild type. Based on signal-to-noise ratio, the chl:P700 ratio was larger than 30,000:1 in F8 and B4, whereas it was 943 (±40):1 in 137c. These PSI-deficient mutants grow photoautotrophically without PSI. PSII is sufficient to drive photosynthetic electron transport from water to the terminal acceptor CO_2 in living cells. The per-photon energy captured by PSII photochemistry at 680 nm (1.8 eV) is energetically sufficient to drive water splitting and the reduction of CO_2, with a maximum energy efficiency of about 68% (*8*). Therefore, although our findings contradict the belief that PSI is essential for oxygenic photosynthesis and for photoautotrophic growth with water as the electron donor, they still obey the laws of thermodynamics.

The role of PSI in photosynthesis could therefore be accessory but beneficial for natural survival. PSI enhanced the rate of photoautotrophic growth by about 30 to 40% in 137c, as compared with the PSI-deficient mutants F8 and B4 (Fig. 1). Furthermore, PSI also conferred resistance to photoinhibition. As demonstrated by a photoautotrophic growth experiment under high light intensity (PAR, 2000 μE m^{-2} s^{-1}; which is equivalent to full sunlight in summer) with air, the mutants grew from an initial population of 6.5×10^4 cells per milliliter to a final culture density of about 8.5×10^5 cells per milliliter in 12 day and night cycles, and 46.6% of the cells became colorless (photobleached), whereas the wild type reached a culture density of 1.72×10^6 cells per milliliter and only 7.8% of the cells were photodamaged. These results indicate that PSI-deficient mutants can grow photoautotrophically even under high light intensity with air, although they have less resistance to photoinhibition than does the wild type.

Photoautotrophic growth similar to that seen with F8 and B4 was observed in initial

experiments using some but not all other nuclear PSI-deficient mutants (mutants were provided by the Duke University *Chlamydomonas* Genetic Center). Mutants ac9 (CC-521), ac80 (CC-544), and F23 (CC-1062) and a chloroplast PSI-deficient mutant, 10-3C (CC-2046), demonstrated photoautotrophic growth, whereas mutants ac-u-g-2-3.7 (CC-703) and ac-215 (CC-1234) did not.

Debate has centered about whether the quantum requirement for photosynthesis is less than or greater than eight photons ($h\nu$) per molecule of O_2 evolved. Some conclude that the minimal quantum requirement is 5 to 6 $h\nu/O_2$ in wild-type green algae (*17, 18*). Such values, if correct, cannot be explained by the Z scheme as it predicts a quantum requirement of at least 8 $h\nu/O_2$. These previously reported quantum requirements may, however, be consistent with the PSII photosynthesis demonstrated in the PSI-deficient mutants of green algae such as F8, B4, ac9, and 10-3C. The minimal quantum requirement for PSII photosynthesis should be 4 $h\nu/O_2$, because PSII photosynthesis uses a single light reaction (PSII) instead of two (PSI and PSII). Pathway studies with the chemical inhibitors 3[3,4-dichlorophenyl]-1,1-dimethylurea, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone, 2-*n*-nonyl-4-hydroxyquinoline-*N*-oxide, and carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone indicated that in PSII photosynthesis, electron flow from PSII to Fd/$NADP^+$ reduction is through the plastoquinone pool and the cytochrome b/f complex (*19*). When both practical energy loss (such as a loss of about 15% excitations in PSII antenna) and involvement of PSI activity (such as PSI cyclic photophosphorylation) are considered, a quantum requirement (4 $h\nu/O_2$) for PSII photosynthesis can explain the reported values of 5 to 6 $h\nu/O_2$ in wild-type green algae (*17, 18*). The previously reported quantum requirement (5 to 6 $h\nu/O_2$) may suggest that PSII photosynthesis can occur even in wild-type algae.

Measurements for many C_3 higher plants have shown a quantum requirement of <8 $h\nu/O_2$, such as 7.67 ± 0.10 $h\nu/O_2$ for *Atriplex littoralis* and 7.69 ± 0.16 $h\nu/O_2$ for *Vicia faba* (*20*). This finding, again, cannot be explained by the Z scheme. Based on high energy consumption in the multiple-cell tissue, estimates have indicated that the minimal quantum requirement for Z scheme photosynthesis would be at least about 10 $h\nu/O_2$ in C_3 higher plants (*14, 21*). Because PSII photosynthesis can have twice the energy efficiency of the Z scheme, the observed quantum requirement in these higher plants can, in principle, be explained by the occurrence of PSII photosynthesis. Therefore, PSII photosynthesis may occur not only in green algae but also in higher plants, and the Z scheme may not be the only mode of oxygenic photosynthesis.

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Role of the *Yersinia pestis* Hemin Storage (*hms*) Locus in the Transmission of Plague by Fleas

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Yersinia pestis, the cause of bubonic plague, is transmitted by the bites of infected fleas. Biological transmission of plague depends on blockage of the foregut of the flea by a mass of plague bacilli. Blockage was found to be dependent on the hemin storage (*hms*) locus. *Yersinia pestis hms* mutants established long-term infection of the flea's midgut but failed to colonize the proventriculus, the site in the foregut where blockage normally develops. Thus, the *hms* locus markedly alters the course of *Y. pestis* infection in its insect vector, leading to a change in blood-feeding behavior and to efficient transmission of plague.

The plague bacillus *Yersinia pestis* persists among certain wild rodent populations in many parts of the world and is transmitted primarily by fleas (*1*). After being ingested in a blood meal, the bacteria multiply in the flea gut and form a mass that can occlude the proventriculus, a spined chamber located between the esophagus and midgut. Such fleas are said to be "blocked" because they are unable to pump blood into their midgut. During persistent but futile attempts to feed on a new host, a blocked flea regurgitates infected blood into the bite site, thus transmitting plague (*2*).

The events after transmission that lead to disease in mammals are well studied, but the molecular and genetic mechanisms by which *Y. pestis* colonizes and blocks its insect host have rarely been addressed (*3, 4*). A pigmented *Y. pestis* phenotype, caused by the storage of exogenous hemin or of Congo red dye in the outer membrane of bacteria at 26°C or lower (*5*), is suited to the hemin-rich, ambient temperature environment of the flea gut. The hemin storage (*hms*) locus responsible for this phenotype consists of *hmsF* and *hmsH*, which encode outer membrane proteins, and *hmsR*, whose product has not been characterized (*6*). Mutation to a nonpigmented phenotype often occurs by deletion of a 102-kb chromosomal segment termed the *pgm* locus that includes *hmsHFR* (*7*), and it has been reported that a nonpigmented *Y. pestis* did not survive in fleas (*8*).

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