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

B. Joseph Hinnebusch,* Robert D. Perry, Tom G. Schwan

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*).

B. J. Hinnebusch and T. G. Schwan, Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT 59840, USA.
R. D. Perry, Department of Microbiology and Immunology, University of Kentucky, Lexington, KY 40536–0084, USA.

*To whom correspondence should be addressed. E-mail: joe_hinnebusch@nih.gov

To investigate the role of this *Y. pestis* genetic locus in the invertebrate host, we infected Oriental rat fleas (*Xenopsylla cheopis*) with pigmented (*hms*$^+$) *Y. pestis* and isogenic nonpigmented (*hms*$^-$) mutants (9). After an infectious blood meal, fleas were monitored for 4 weeks for mortality and blockage (*10*) (Fig. 1). Only fleas infected with *hms*$^+$ *Y. pestis* developed blockage, with accompanying high mortality (Table 1). The isogenic *hms*$^-$ mutants were completely unable to block fleas and did not increase flea mortality during the 4-week observation period. These results indicate that the *hms* locus is required for *Y. pestis* to cause blockage of fleas. Other genes in the 102-kb *pgm* locus besides *hmsHFR* are not necessary for blockage, because *Y. pestis* strain KIM6, a Δ*pgm* mutant, regained the ability to block fleas when complemented with pHMS1, a low copy number recombinant plasmid containing *hmsHFR*. An *hms* polar mutant with a single transposon insertion in *hmsH* (6) was also unable to block.

A possible explanation for the inability of *hms*$^-$ *Y. pestis* to block is that they are rapidly eliminated by the flea and never establish infection. We therefore compared the fates of *hms*$^+$ and *hms*$^-$ *Y. pestis* after ingestion by fleas (Fig. 2). By 1 week after the infectious blood meal, fleas that ingested either *hms*$^+$ or *hms*$^-$ strains segregated into two groups: those cleared of infection and those that contained 10^3 to 10^6 bacteria. The tendency for some fleas to rid themselves of infection after feeding on even highly septicemic blood has been noted previously (*1*, *11*). This dichotomy was even more pronounced in samples of fleas that were maintained for 4 weeks after infection (Fig. 2, B and C). Even after heavily infected blocked fleas and fleas that died during the 4 weeks were excluded, 70% of surviving fleas originally infected with *hms*$^+$ *Y. pestis* strains contained 10^4 to 10^6 viable bacteria. Although fleas infected with *hms*$^-$ strains never developed blockage and were more likely to clear the bacteria than were fleas infected with *hms*$^+$ strains, 50% still contained 10^3 to 10^5 *hms*$^-$ *Y. pestis* 1 week after the infectious blood meal, with the same percentage being heavily infected after 4 weeks (Fig. 2). Thus, the failure of *hms*$^-$ *Y. pestis* to block fleas cannot be attributed to an inability to colonize the flea's midgut.

To better see where bacteria locate and develop in the flea gut, we next infected *X. cheopis* with *hms*$^+$ or *hms*$^-$ *Y. pestis* express-

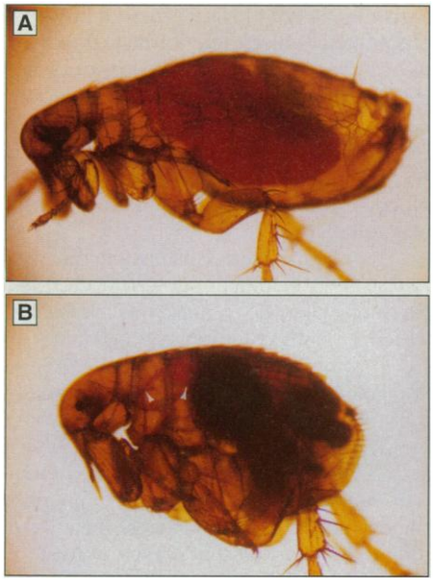

Fig. 1. Blocked and unblocked *X. cheopis* fleas immediately after a blood meal. (**A**) Uninfected normal male with fresh blood in midgut. (**B**) *Yersinia pestis*–infected blocked female. The presence of fresh blood in the esophagus only (indicated by arrowheads) and not in the midgut, which contains dark-colored digestion products of previous blood meals, is diagnostic of a blocked flea.

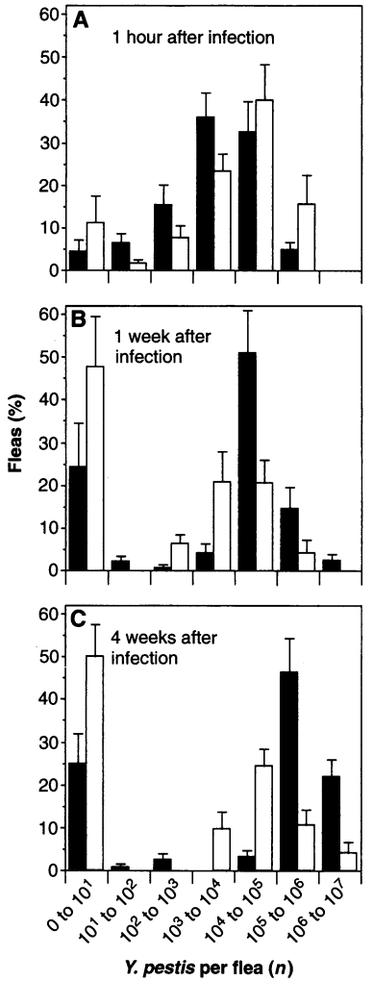

Fig. 2. Frequency distributions of percentages of *X. cheopis* fleas harboring different numbers of *hms*$^+$ (solid bars) or *hms*$^-$ (open bars) *Y. pestis* at successive time intervals after a single infectious blood meal. Within an hour after ingesting blood containing 5×10^8 *Y. pestis* colony-forming units (CFUs) per milliliter (*9*), 20 female fleas were placed at −70°C. The number of *Y. pestis* CFUs per individual flea was later determined by plate count (*21*) to assess the initial infectious dose (**A**). To follow the development of infection, the number of *Y. pestis* per flea was also determined for samples of 20 female fleas maintained for 1 week (**B**) and for 4 weeks (**C**) after the infectious blood meal. Included are the results of three experiments each for fleas infected with *Y. pestis hms*$^+$ strains KIM6+ and KIM6 (pHMS1) and with the isogenic *hms*$^-$ mutant strains KIM6 and KIM6+ (*hmsH*::minikan).

Table 1. Mortality and blockage of *X. cheopis* fleas during a 4-week period after infection with *hms*$^+$ *or hms*$^-$ *Y. pestis*. Equal numbers of female and male fleas were infected artificially, except where noted, and were subsequently fed twice weekly on mice and monitored for blockage and mortality (*9*, *10*). Results of replicate experiments are shown. *Yersinia pestis* strain 195/P was isolated from a human plague patient in India and is fully virulent. The isogenic *hms*$^-$ derivative contains a small deletion or point mutation in *hmsR* (*23*). *Yersinia pestis* strain KIM6+ originated from a human in Iran. It lacks the 70-kb *Yersinia* plasmid that is required for virulence in mammals but not for blockage of fleas (*7*, *22*). A spontaneous deletion of the 102-kb *pgm* locus (Δ*pgm*) generated strain KIM6 (*7*). Plasmid pHMS1 contains *hmsHFR*; complementation of KIM6 with this plasmid restores pigmentation. A second isogenic nonpigmented mutant of KIM6+ was produced by transposon insertion into *hmsH* (*6*). Plus signs indicate pigmented colonies; minus signs indicate colorless colonies.

Fleas infected with	Hms*	Fleas blocked (%)	Flea mortality (%)	Fleas (*n*)
Y. pestis 195/P†	+	50	74	100
Y. pestis 195/P	+	45	72	57
		50	52	95
		32	42	100
Y. pestis 195/P (*hmsR*$^-$)	−	0	8	52
		0	10	78
		0	5	108
Y. pestis KIM6$^+$	+	24	49	100
		38	56	100
		34	33	103
Y. pestis KIM6 (Δ*pgm*)	−	0	4	104
		0	1	104
		0	7	83
Y. pestis KIM6 (Δ*pgm*, pHMS1)	+	42	41	104
		32	42	99
		37	56	101
Y. pestis KIM6$^+$ (*hmsH*::minikan)	−	0	20	102
		0	5	101
		0	23	103
No bacteria		0	13	99
		0	4	95
		0	20	88

*Phenotype of *Y. pestis* strain on Congo red agar plates. †Fleas infected naturally by feeding on a bacteremic mouse.

ing the gene encoding green fluorescent protein (GFP) (*12*). Visualization required removal of the flea's digestive tract, so temporal development of infection in individual fleas could not be directly observed. What follows is a composite description based on weekly examinations of 5 to 10 male and female fleas for 8 weeks.

During the first week of infection, both *hms*$^+$ and *hms*$^-$ bacteria coalesced into one or more aggregates in the midgut that were completely contained within dense masses of what appeared to be agglutinated, brown-pigmented, red blood cell remnants. Because of this covering, the bacteria were visible only by fluorescence microscopy. The masses varied in size and shape and were either free-floating in the flea's midgut or partially anchored in the proventricular spines. In keeping with the quantitative data (Fig. 2), infection appeared to be "all or nothing," with midguts containing either large masses or no bacteria. Large brown masses in the midgut are virtually pathognomonic of *Y. pestis* infection in the flea and were described in the classic work of Bacot and Martin (*2*) and others (*3*, *11*).

After the first week, a fundamental difference became apparent between the infections produced by the two types of bacteria. Whereas *hms*$^-$ mutants remained confined to the midgut, *hms*$^+$ bacteria had spread to the proventriculus in many fleas. Eventually the proventriculus became packed with plague bacilli, which is indicative of a blocked flea (Fig. 3). In contrast, *hms*$^-$ bacteria did not colonize the proventriculus. Small clusters of *hms*$^-$ bacteria were occasionally seen among the proventricular spines, but they appeared to be unable to persist at that site. One or more large brown masses packed with *hms*$^-$ bacteria were, however, seen in the midguts of fleas throughout the 8-week observation period, with no apparent morbidity to the fleas.

The *hms* gene products thus explicitly enable *Y. pestis* to colonize the proventriculus. Nonspecific functions such as resistance to the proteolytic environment of the flea's digestive tract or acquisition of nutritional iron can be ruled out. The proventriculus, unlike the midgut, is lined with cuticle, the specialized material that composes the arthropod exoskeleton. Biogenesis and sclerotization of insect cuticle use phenoloxidase-generated cytotoxic intermediates in pathways that are shared by an arm of insect immunity (*13*). Cuticularized insect epithelium has also been shown to secrete antibacterial peptides upon damage and exposure to bacteria (*14*). We compared the in vitro sensitivity of *hms*$^+$ and *hms*$^-$ *Y. pestis* to cuticle sclerotization intermediates and to Cecropin A, but found no differences. In addition, both types of bacteria remained viable 1 week after injection into the flea hemocoel (*15*), which suggests that the *hms* genes do not confer increased resistance to insect defense mechanisms.

Rhythmic contractions of the proventriculus as blood is pumped through it during feeding produce a threshing action of the hard cuticularized spines that would disrupt an occluding mass. A cell surface change of *Y. pestis* that results from expression of the *hms* genes likely serves to defeat these forces. *hms*$^+$ *Y. pestis* in vitro are extremely aggregative and hydrophobic (*5*), whereas isogenic *hms*$^-$ mutants are hydrophilic with no autoaggregation. In certain other enterobacteriaceae, hydrophobic autoaggregative growth and Congo red binding correlate with expression of thin fimbriae that mediate adherence to fibronectin and other basement membrane proteins (*16*). We detected no differences in adherence of *hms*$^+$ or *hms*$^-$ *Y. pestis* to the cuticle lining of the proventriculus (*17*). Nevertheless, as suggested by Bibikova (*18*), the cohesive aggregates of wild-type *Y. pestis* may remain enmeshed in a pulsating proventricular valve. Continued growth of a partially lodged bacterial mass during quiescent periods would lead to further consolidation and eventual occlusion of the proventriculus. Masses of *hms*$^-$ mutants may fail to colonize the proventriculus because, being less cohesive, they are disrupted and flushed back into the midgut during feeding.

Our results indicate that *Y. pestis* transmission by a principal rat flea vector depends on the *hms* genes, because only blocked fleas transmit plague efficiently (*1*). It is the first genetic locus of an arthropod-borne bacterium that has been shown to be required for transmission-competent infection in the vector. The *hms* locus does not appear to be required in mammals because the nonpigmented *hmsR*$^-$ *Y. pestis* used in this study (Table 1) is virulent for mice (*19*). Whatever their mechanism of action, the *hms* genes effectively change *Y. pestis* from a harmless commensal in the flea midgut to one that can block the foregut, leading to starvation of the flea that prompts persistent, aggressive attempts to feed (*1*, *2*). This results in transmission of the plague bacillus and the eventual death of the flea. The *hms* locus could therefore be described as a *Y. pestis* virulence factor in the flea. From the anthropocentric viewpoint of plague epidemiology, however, it may more aptly be termed a transmission factor.

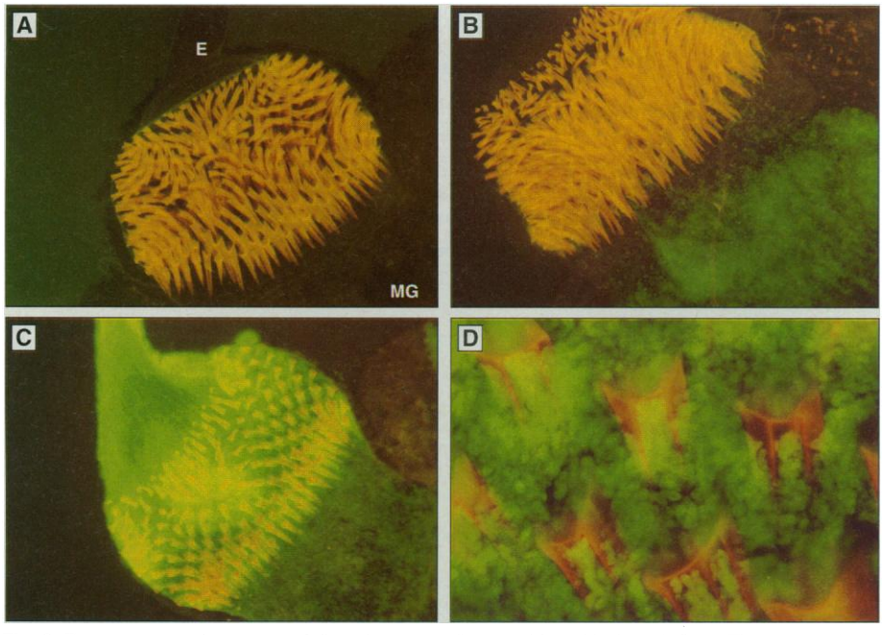

Fig. 3. Fluorescence microscopy of digestive tracts dissected from *X. cheopis* fleas. The proventriculus connects the esophagus (E) to the midgut (MG) and is armed with spines that autofluoresce yellow-orange because they, like the insect's exoskeleton, are covered with cuticle. (**A**) Uninfected flea. (**B**) Flea infected 4 weeks previously with *hms*$^-$ *Y. pestis* strain KIM6 expressing GFP. (**C**) Blocked flea infected with *hms*$^+$ *Y. pestis* strain KIM6+ expressing GFP. In the midguts of infected fleas, both *hms*$^+$ and *hms*$^-$ bacteria were only discernable with the use of fluorescence because they were covered by agglutinated red blood cell remnants. Original magnification, ×160. (**D**) Close-up of proventriculus from a blocked flea. The lumen is filled with a bacterial mass, shown here at the base of the hexagonal proventricular spines. Original magnification, ×1000.

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10. Blockage was determined by microscopic examination of each flea immediately after twice-weekly feedings on normal mice. Fleas that contained fresh blood only in the esophagus, anterior to the proventriculus and midgut, were defined as blocked (Fig. 1). The interval between infection and the appearance of blockage (the extrinsic incubation period) ranged from 5 to 51 days, with a median of 18 days. Male fleas tended to block sooner than females. Blocked fleas made prolonged, persistent attempts to feed, died within a week after development of the block, and contained more than 10^6 *Y. pestis* bacteria. These features are consistent with previous descriptions of the blockage phenomenon in *X. cheopis* (*1*, *11*).
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Modification of Phytohormone Response by a Peptide Encoded by *ENOD40* of Legumes and a Nonlegume

Karin van de Sande,* Katharina Pawlowski,* Inge Czaja, Ursula Wieneke, Jeff Schell, Jürgen Schmidt, Richard Walden, Martha Matvienko,* Joan Wellink, Ab van Kammen, Henk Franssen, Ton Bisseling†

The gene *ENOD40* is expressed during early stages of legume nodule development. A homolog was isolated from tobacco, which, as does *ENOD40* from legumes, encodes an oligopeptide of about 10 amino acids. In tobacco protoplasts, these peptides change the response to auxin at concentrations as low as 10^{-12} to 10^{-16} M. The peptides encoded by *ENOD40* appear to act as plant growth regulators.

Legume nodule organogenesis is initiated by local dedifferentiation of root cortical cells activated by rhizobial Nod factors (*1*, *2*). Nod factors probably trigger cell divisions by inducing a local change of the auxin/cytokinin ratio (*3*). The expression of a few nodule-specific plant (nodulin) genes is induced by Nod factors (*4*, *5*). One of these genes, *ENOD40*, is first expressed in the root pericycle opposite to the nodule primordium (*4*, *6*–*8*). Expression precedes the induction of cortical cell divisions (*8*), which suggests that *ENOD40* may play a role in changing the response to phytohormones. To test this, we used tobacco as a model system.

To investigate *ENOD40* action, we transformed tobacco plants (*9*) with the construct 40-2/1-448, representing 448 base pairs (bp) of soybean *GmENOD40-2* under the control of the cauliflower mosaic virus (CaMV) 35S promoter (Fig. 1) (*10*, *11*). Thirty percent of transgenic F_1 plants had one or two adventitious shoots at the base of the main shoot, as compared with none in untransformed tobacco plants (Fig. 2A). This suggests reduced apical dominance, raising the possibility that the transgenic plants were changed in terms of auxin me-

K. van de Sande and K. Pawlowski, Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné-Weg 10, 50829 Köln, Germany, and Department of Molecular Biology, Agricultural University, 6703 HA Wageningen, Netherlands.
I. Czaja, U. Wieneke, J. Schell, J. Schmidt, R. Walden, Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné-Weg 10, 50829 Köln, Germany.
M. Matvienko, J. Wellink, A. van Kammen, H. Franssen, T. Bisseling, Department of Molecular Biology, Agricultural University, 6703 HA Wageningen, Netherlands.

*These authors contributed equally to this work.
†To whom correspondence should be addressed.