could potentially reassort with LAC virus, infect humans much more frequently (*7*). Since LAC virus is sympatric in portions of its distribution with certain of these bunyaviruses, it is possible that reassortment of genomes could occur, resulting in serious epidemiologic consequences.

Dual infection of mosquitoes by interrupted feeding may enhance possibilities for evolution of bunyaviruses in vectors. Although *A. triseriatus* is a relatively long-lived mosquito, the number of gonadotrophic cycles is limited (*8*). Thus, chances for dual infection, segment reassortment, and transmission of new virus genotypes would be more likely if dual infection occurred during the first gonadotrophic cycle. Opportunities for generation of new virus genotypes in mosquitoes infected with the first virus in one gonadotrophic cycle and the second virus during a subsequent cycle would be limited both by interference and by the life history of the mosquito. Most females would not live long enough to successfully transmit newly generated reassortant viruses to susceptible vertebrates. Virtually all mosquitoes became dually infected in the interrupted feeding studies (Table 3). In only about 25 percent of the specimens did both viruses infect the same cell, yielding reassortant viruses. Perhaps different virus combinations, different vector mosquitoes, or different experimental conditions, such as a longer incubation period or additional gonadotrophic cycles, would yield more reassortant viruses. Alternatively, there may be genetic constraints on the generation of reassortant viruses in mosquitoes.

These studies demonstrate the potential for RNA segment reassortment of bunyaviruses in dually infected vectors. The frequency of these events in more biologically relevant systems (such as heterologous wt virus crosses) and the significance of these events in nature remain to be determined.

References and Notes

1. D. Bishop and R. Shope, in *Comprehensive Virology 14*, H. Fraenkel-Conrat and R. Wagner, Eds. (Plenum, New York, 1979), pp. 1–156; F. Fuller, A. Bhown, D. Bishop, *J. Gen. Virol.* **64**, 1705 (1983); F. Fuller and D. Bishop, *J. Virol.* **41**, 643 (1982); D. Bishop *et al.*, in *Segmented Negative Strand Viruses, Arenaviruses, Bunyaviruses, and Orthomyxoviruses*, R. Compans and D. Bishop, Eds. (Academic Press, New York, 1984), pp. 3–11.
2. P. Palese and J. F. Young, *Science* **215**, 1468 (1982); W. Laver, R. Webster, C. Chu, *J. Infect. Dis.* **149**, 108 (1984).
3. B. Beaty, E. Rozhon, P. Gensemer, D. Bishop, *Virology* **111**, 662 (1981); R. Klimas *et al.*, *Am. J. Epidemiol.* **114**, 112 (1981); C. Pringle, J. Lees, W. Clark, R. Elliot, *Virology* **135**, 244 (1984).
4. B. Beaty, D. Bishop, M. Gay, F. Fuller, *Virology* **127**, 83 (1983).
5. J. Edman and A. Downe, *Mosq. News* **24**, 154 (1964); M. Klowden and A. Lea, *Am. J. Trop. Med. Hyg.* **27**, 827 (1978); L. Magnarelli, *J. Med. Entomol.* **13**, 445 (1977); C. Mitchell and K. Millan, *ibid.* **18**, 249 (1981).
6. B. J. Beaty *et al.*, *Science* **211**, 1433 (1981); B. Beaty, B. Miller, R. Shope, E. Rozhon, D. Bishop, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1295 (1982); R. Shope, G. Tignor, E. Rozhon, D. Bishop, in *Replication of Negative Strand Viruses*, D. Bishop and R. Compans, Eds. (Elsevier, New York, 1981), pp. 147–152; G. Tignor, T. Burrage, A. Smith, R. Shope, in *California Serogroup Viruses*, C. Calisher and W. Thompson, Eds. (Liss, New York, 1983), pp. 129–138; C. Seymour, T. Amundson, T. Yuill, D. Bishop, *Am. J. Trop. Med. Hyg.* **32**, 1147 (1983); R. Janssen, F. Gonzalez-Scarano, N. Nathanson, *Lab. Invest.* **50**, 447 (1984).
7. J. LeDuc, *J. Med. Entomol.* **16**, 1 (1979); T. Monath, *Bull. WHO* **57**, 513 (1979); P. Grimstad, *Adv. Virus Res.* **28**, 357 (1983).
8. M. Sinsko and G. Craig, *J. Med. Entomol.* **15**, 89 (1979); L. Haramis and A. Woodbridge, *ibid.* **20**, 391 (1983); G. DeFoliart, in *California Serogroup Viruses*, C. Calisher and W. Thompson, Eds. (Liss, New York, 1983), pp. 89–104.

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Cyanobacterial Light-Harvesting Complex Subunits Encoded in Two Red Light–Induced Transcripts

Abstract. *The major light-harvesting complex in cyanobacteria and red algae, the phycobilisome, is composed of chromophoric and nonchromophoric polypeptides. Two linked genes encoding major chromophoric components, the polypeptide subunits of phycocyanin, were isolated from the cyanobacterium* Fremyella diplosiphon. *Transcripts from this phycocyanin subunit gene cluster were present as major species in the cyanobacterium grown in red light, but not in cultures maintained in green light. The genes for the subunits of the red light–induced phycocyanin were transcribed together (β-phycocyanin followed by α-phycocyanin) on two messenger RNA species; one contained 1600 bases while the other had 3800 bases. The latter, which encompassed the smaller transcript, contained additional sequences extending from the 3′ end of the coding region of the α-phycocyanin gene. It may encode other light-induced components of the phycobilisome. Since phycocyanin, which effectively absorbs red light, becomes a dominant constituent of the phycobilisome in red light, these different levels may reflect an important adaptive mechanism of these organisms to their environment.*

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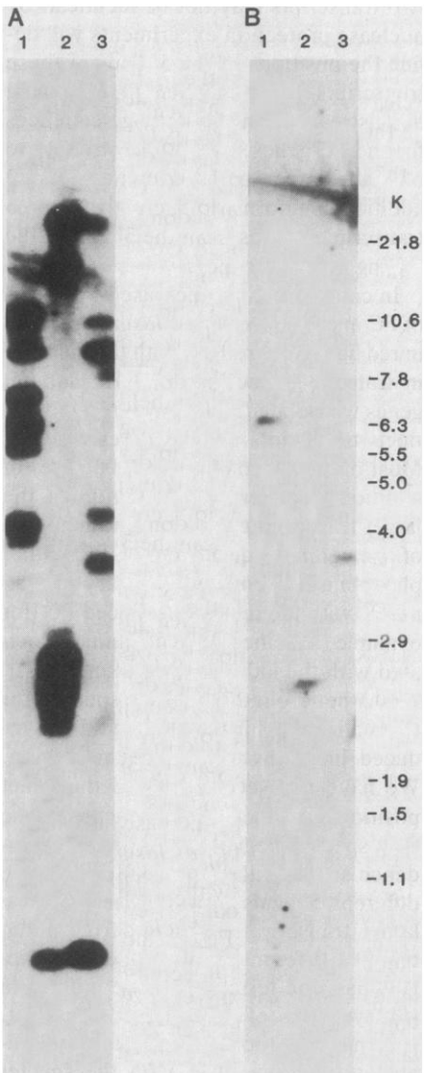
Phycobilisomes are major light-harvesting complexes in prokaryotic cyanobacteria and eukaryotic red algae and may constitute 50 percent of soluble cellular protein (*1*). Chromophoric proteins (phycobiliproteins) present in the two domains of this complex, the core which is directly anchored to the photosynthetic membranes and the rods which are attached to the core (*2, 3*), serve to absorb light energy and transfer this energy to the photosynthetic reaction center (*4*). The three major chromophoric proteins are phycocyanin (PC), allophycocyanin (APC), and phycoerythrin (PE), each of which is composed of two related subunits, α and β. In addition to these polypeptides, specific nonchromophoric linker polypeptides mediate the association between the different phycobiliproteins. The constituents of the phycobilisome in many cyanobacteria vary to optimize the absorption of prevalent wavelengths of light (*1, 5*), a phenomenon termed complementary chromatic adaptation (*1*). Evidence obtained with inhibitors of transcription (*6*) suggests that a change in light quality triggers an alteration in the transcription of phycobiliprotein genes. However, little is known about the genes encoding phycobilisome polypeptides and how their transcription is controlled.

We recently isolated and characterized the genes for four phycobiliprotein subunits (α- and β-PC and α- and β-APC) from the plastid genome of the eukaryotic alga *Cyanophora paradoxa* (*7, 8*). Others have isolated PC genes from the cyanobacterium *Agmenellum quadruplicatum* (*9, 10*). In eukaryotic algae, the subunit genes for PC and APC are each transcribed on a dicistronic messenger RNA (mRNA) (*8*), as is the case for the PC subunit genes in *A. quadruplicatum* (*9*). The PC subunit genes of *C. paradoxa* (*7*) were used in heterologous hybridizations to isolate analogous genes in the cyanobacterium *Fremyella diplosiphon*. Figure 1 shows a Southern hybridization in which two fragments generated from the plastid-encoded PC subunits were hybridized to genomic DNA from *F. diplosiphon* digested with various restriction enzymes. One fragment used was a 0.8-kilobase (kb) sequence (*8*) encoding almost all of β-PC and the amino terminal third of α-PC. A second fragment, generated by Sau3A digestion of the insert from pCPC2368 (*7*), contained 63 base pairs (bp) encoding the con-

served, carboxyl terminal chromophore binding site of β-PC, and showed specificity for β-PC under the hybridization conditions used.

The 0.8-kb fragment hybridized, at moderate stringency (approximately 40 percent mismatch allowed), to several fragments of *F. diplosiphon* genomic DNA in each restriction digest (Eco RI, Pst I, and Hind III) (Fig. 1A). Since amino acid composition data suggest that there are at least two different sets of PC polypeptides [one constitutively expressed and the other expressed only in red light (*11*)] and since linkage of α- and β-PC subunit genes has been demonstrated (*9, 10*), a minimum of two hybridizing fragments in each digest are likely to encode PC subunits. In addition, the phycobiliprotein subunits are encoded by a family of genes that probably arose from a single ancestral gene (*12*); therefore, the other restriction fragments that hybridized to the 0.8-kb sequence probably encode other phycobiliproteins [α- and β-subunits of APC, perhaps multiple species of α- and β-PE as well as other minor components (*13–15*)]. Under the same conditions of stringency, the sequence covering the conserved chromophore binding site of β-PC from *C. paradoxa* hybridized to only one fragment in each digest (6.6-kb Eco RI fragment, 2.6-kb Pst I fragment, and 3.8-kb Hind III fragment) (Fig. 1B). The single hybridizing species in each digest corresponded to one of the strongly hybridizing fragments observed when the 0.8-kb sequence was used (Fig. 1, A and B). Since a single species hybridized in each digest, only one of the β-PC genes of *F. diplosiphon* showed strong homology to the *C. paradoxa* region encoding the carboxyl terminal chromophore binding site of β-PC. When the stringency of hybridization was decreased (approximately 50 percent mismatch), additional sequences hybridized to the 63-bp probe.

To isolate the PC genes hybridizing to the 63-bp sequence, we digested *F. diplosiphon* genomic DNA with Hind III, fractionated it on an agarose gel, and cloned fragments of approximately 3.8



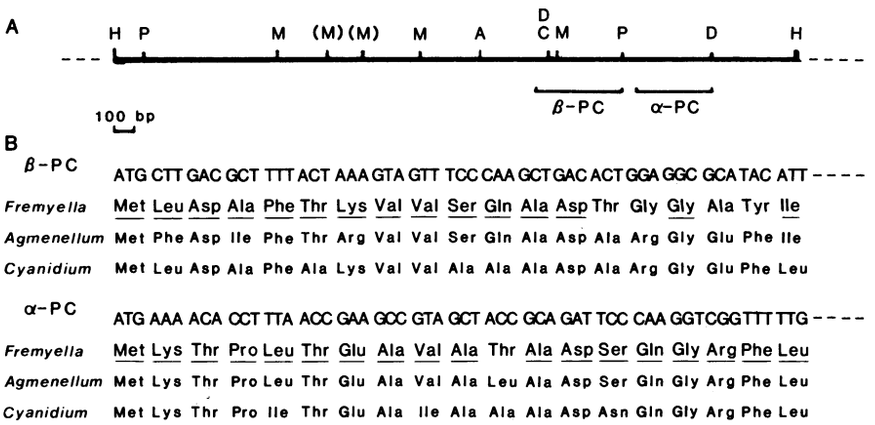


Fig. 1 (left). Hybridizations of total genomic *F. diplosiphon* DNA to heterologous PC sequences. Genomic DNA (~1 μg), isolated (*18*) from *F. diplosiphon* [UTEX 481 grown on Gorham's medium (*19*)], was digested with restriction endonucleases, electrophoresed on a 0.8 percent agarose–tris acetate–EDTA (TEA) gel (*20*), and transferred to nitrocellulose. The enzymes used were Eco RI (lanes 1), Pst I (lanes 2), and Hind III (lanes 3). (A) Hybridization to a nick-translated, 0.8-kb fragment from *C. paradoxa* [Eco RI–Pst I fragment from pCPC4067 (*7*)] encoding the amino terminal third of α-PC from *C. paradoxa* and all but 17 amino terminal amino acids of β-PC. (B) Hybridization to a 63-bp *C. paradoxa* fragment [containing 33 bp encoding the conserved carboxyl terminal chromophore binding site of β-PC and 15 bp on either side (*7*)], labeled by fill-in of protruding Sau3A ends with the Klenow fragment of DNA polymerase I. Hybridizations (*7*) were done at 55°C (15 to 18 hours, 5×10^5 Cerenkov count/min per filter). The 0.8-kb fragment was hydrolyzed to 300 to 400 bp by boiling in 0.5*M* NaOH for 5 minutes. After hybridization, the filters were washed three times [10 minutes each time with 0.01*M* PO_4^- buffer (*p*H 7.0), 0.02*M* EDTA, and 0.1 percent sodium dodecyl sulfate] at room temperature and exposed to Kodak XAR-5 film (−80°C for 24 hours). Molecular weight markers are Eco RI restriction fragments of pRR12 (*7*). Fig. 2 (above). (A) Restriction enzyme map of the 3.8-kb Hind III insert from pFD126. Total genomic *F. diplosiphon* DNA digested with Hind III was fractionated on a 0.8 percent agarose-TEA gel (*20*) and fragments of approximately 3.8 kb were electrophoresed onto NA-45 paper (Schleicher & Schuell) and eluted in accordance with the manufacturer's specifications. Fragments were ligated into pUC8 and transformed colonies were screened by colony hybridization (*21*) with the *C. paradoxa* β-PC gene (0.8 kb; see legend to Fig. 1). The insert DNA from a positively hybridizing clone (pFD126) was mapped with restriction endonucleases [Hind III (H), Pst I (P), Msp I (M), Aha III (A), Dde I (D), and Cla I (C)]. The location of the Msp I site in parentheses (M) is not known but is in one of the two indicated positions. Hybridizations of *C. paradoxa* DNA fragments encoding β-PC (0.8 kb; see legend to Fig. 1) or α-PC (encoding all of α-PC, the carboxyl terminal 31 amino acids of β-PC, and approximately 800 bp at the 3′ end of the structural gene for α-PC) to DNA from the 3.8-kb insert indicated the probable positions of the genes for the α and β subunits. These assignments were confirmed by sequencing. The direction of transcription for the PC genes is left to right. (B) Nucleotide and predicted amino acid sequences of the 5′ ends of the PC genes in the 3.8-kb Hind III insert of pFD126. The nucleotide sequence encoding the amino terminus of the β-PC gene was determined (*22*) after labeling at the Cla I site with polynucleotide kinase or after fill-in with the Klenow fragment of DNA polymerase I. The nucleotide sequence encoding the amino terminus of α-PC was determined after ligating the Pst I and Hind III fragment (1.0 kb) of the 3.8-kb insert of pFD126 into the M13 vector mp19 (*24*). The sequence encoding β-PC begins 67 bp to the left of the Cla I site and ends 11 bp to the right of the Pst I site. The α-PC gene begins 84 bp to the right of the Pst I site and ends just to the left of the Dde I site. The predicted amino acid sequence is shown after *Fremyella*. The amino acid sequence determined from the nucleotide sequence of *A. quadruplicatum* (*9, 10*) is shown after *Agmenellum*; the amino acid sequence derived from purified PC subunits of *C. caldarium* is presented after *Cyanidium* (*16*). Underlined residues in the *Fremyella* sequence are identical to the *Agmenellum* or *Cyanidium* sequence at that position.

kb into pUC8. Figure 2 shows a restriction map for a 3.8-kb insert isolated from a colony (pFD126) hybridizing to the 0.8-kb fragment. The positions of the β- and α-PC genes were determined by heterologous hybridizations with the *C. paradoxa* genes; the probes used were the 0.8-kb fragment, which encodes the conserved regions of β-PC, and a 1.4-kb fragment from pCPC2368 (*7*), which encodes the conserved regions of α-PC. The positions of the α- and β-PC genes were confirmed by sequence analysis of the 3.8-kb insert. The *F. diplosiphon* nucleotide sequences encoding the first 19 amino acids of α- and β-PC are presented in Fig. 2B. The predicted amino acid sequences of these subunits from *F. diplosiphon* compared to analogous sequences from *A. quadruplicatum* (*9, 10*) and from the eukaryotic alga *Cyanidium caldarium* (*16*) are highly homologous.

Since *F. diplosiphon* modulates PC levels during complementary chromatic adaptation (*1*), we examined the effect of different illumination conditions on transcripts homologous to the 3.8-kb insert. Northern analyses of RNA isolated from cultures maintained in red or green light are presented in Fig. 3. Restriction fragments (denoted A, B, C, and D) generated from the 3.8-kb Hind III insert were hybridized under relatively stringent conditions (less than 30 percent mismatch) to the RNA. The locations of fragments A to D with respect to the coding sequences of the PC subunit genes are presented in the upper part of Fig. 3. A Dde I fragment (fragment C) encoding all of α-PC and all but the amino terminus of β-PC hybridized strongly to two mRNA species, 1.6 and 3.8 kb in size, present only in RNA from cells grown in red light, not in RNA from cells grown in green light. The smaller transcript is only slightly larger than the transcripts encoding α- and β-PC in *A. quadruplicatum* (*9*) and *C. paradoxa* (*8*). The faintly hybridizing sequences (X, Y, and Z in Fig. 3) present in all the hybridizations correspond to the positions of ribosomal RNA species. When total *F. diplosiphon* RNA was hybridized to a 2.4-kb fragment (fragment B) containing the 5′ end of the β-PC gene and additional upstream sequences, both the 1.6- and 3.8-kb transcripts hybridized. However, when a 2.0-kb fragment (fragment A) commencing approximately 400 bp upstream of the coding region for β-PC was used, there was no detectable hybridization to the 1.6-kb transcript and low-level hybridization to the 3.8-kb transcript. Since the DNA species were in excess, were hydrolyzed, and were of similar specific activities, the relative intensities of hybridization in fragments A to D indicate the extent of the DNA sequences homologous to the RNA. Therefore, only a small region of fragment A is homologous to the 3.8-kb transcript, suggesting that the larger transcript must begin close to the Aha III cleavage site. Since the same fragment did not show detectable hybridization to the 1.6-kb transcript, the 5′ ends of the two transcripts may not be identical. S1 nuclease protection experiments will define the positions of the 5′ ends of these transcripts. Since fragment D, extending downstream from the coding sequences for the PC genes, hybridized strongly to only the larger transcript, most of the additional information on the 3.8-kb transcript extends from the 3′ end of the coding region for α-PC.

In conclusion, the increased synthesis of PC in cultures of *F. diplosiphon* grown in red light is correlated with the increase in transcripts encoding the PC subunit genes we isolated. Furthermore, two distinct transcripts encode these genes. Most of the approximately 2000 bases of additional sequence information in the larger transcript extends from the 3′ end of the coding sequence for α-PC. Other phycobilisome constituents vary coordinately with the red light–induced PC [for example, specific linker proteins associated with the induced PC subunits (*11*)]. A synthetic oligonucleotide specific for the amino terminus of a red light–induced linker hybridizes to this region. We have not yet determined the exact positions at which transcription of these two species begins; however, the evidence suggests that the transcripts have different 5′ ends. The synthesis of the larger transcript may result from initiation at a different start site and transcription past the termination site utilized in the synthesis of the smaller transcript. Alternatively, both transcripts may initiate at the same site, with the smaller transcript being formed by degradation or processing of the larger transcript. Differential stability of portions of a transcript encoding photosynthetic genes in *Rhodopseudomonas capsulata* has been proposed to account for differences in levels of gene products encoded in these transcripts (*17*).

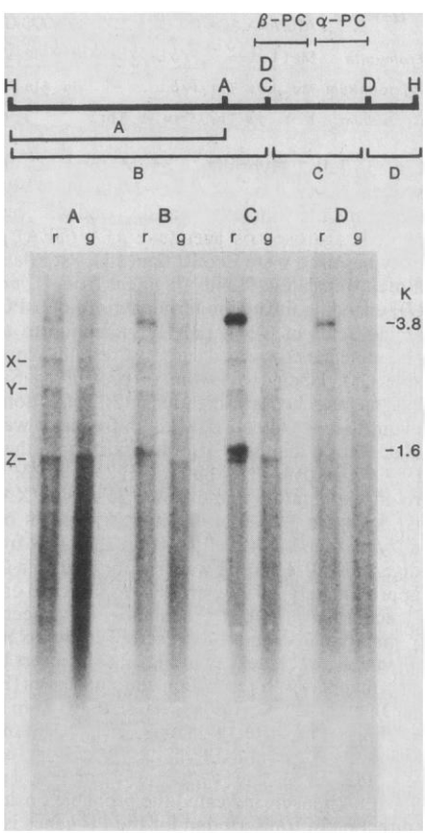


Fig. 3. Hybridization of restriction fragments from the 3.8-kb insert of pFD126 to RNA isolated from cultures of *F. diplosiphon* grown in red and green light. Lanes containing RNA isolated from cultures grown in red or green light are indicated by r and g, respectively. DNA fragments used to hybridize to immobilized RNA are indicated by bars below the appropriate restriction sites on the map. Positions of the genes encoding α- and β-PC are shown and the direction of transcription is left to right. The faintly hybridizing fragments, designated X, Y, and Z, correspond to the positions of ribosomal RNA species. [X is the 23*S* species, Y is a cleavage product of X, and Z is the 16*S* species. When RNA from both cultures were hybridized to an *Escherichia coli* clone (*25*) encoding the 23*S*, 16*S*, and 5*S* ribosomal DNA species at 65°C, bands X, Y, and Z hybridized very strongly.] RNA from cultures grown under red or green illumination (~50 μE m^{-2} sec^{-1}) was isolated by sequential extraction. Cultures (approximately 200 ml) in mid-log phase were collected by vacuum filtration on Whatman 1-mm filter disks and rapidly immersed in 15 ml of 0.1*M* tris-HCl (*p*H 6.5) containing 6*M* guanidine-HCl, 0.01*M* dithiothreitol, and 1 percent *N*-lauroylsarcosine and extracted (*26*). Extraction with phenol and sodium dodecyl sulfate followed (*27*). The purified RNA (2 μg per lane) was electrophoresed in a 1.5 percent agarose gel at 150 V in 1× MEN buffer [0.02*M* *N*-morpholinopropanesulfonic acid (*p*H 7.0), 0.005*M* sodium acetate, and 0.001*M* EDTA (*20*)] and transferred to nitrocellulose without further treatment (*28*). Molecular weight markers (λ digested with Hind III, pBR322 digested with Hpa II) were treated like the RNA samples before loading onto the gel. The nitrocellulose was baked for 3 hours in a vacuum at 80°C. Hybridizations with the nick-translated DNA fragments A, B, C, and D (boiled for 5 minutes in 0.5*M* NaOH) were carried out as described in the legend to Fig. 1 (except at 65°C) (1.5 × 10^6 Cerenkov count/min per filter). The filters were washed and exposed to Kodak XAR-5 film. The specific activity of the probes used was approximately 2.5 × 10^8 count/min per microgram of DNA.

References and Notes

1. L. Bogorad, *Annu. Rev. Plant Physiol.* **26**, 369 (1975).
2. E. Gantt, *ibid.* **32**, 327 (1981).
3. A. N. Glazer, *Annu. Rev. Microbiol.* **36**, 171 (1982).
4. R. T. Wang, C. L. R. Stevens, J. Myers, *Photochem. Photobiol.* **25**, 103 (1977).
5. N. Tandeau de Marsac, *J. Bacteriol.* **130**, 82 (1977).
6. S. Gendel, I. Ohad, L. Bogorad, *Plant Physiol.* **64**, 786 (1979).
7. P. G. Lemaux and A. R. Grossman, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4100 (1984).
8. ———, *EMBO J.* **4**, 1911 (1985).
9. T. J. Pilot and J. L. Fox, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6983 (1984).
10. R. de Lorimier *et al.*, *ibid.*, p. 7946.
11. D. A. Bryant and G. Cohen-Bazire, *Eur. J. Biochem.* **119**, 415 (1981).
12. A. N. Glazer, *Mol. Cell. Biochem.* **18**, 125 (1977).
13. ——— and C. S. Hixson, *J. Biol. Chem.* **252**, 32 (1977).
14. A. C. Ley, W. L. Butler, D. A. Bryant, A. N. Glazer, *Plant Physiol.* **59**, 974 (1977).
15. A. N. Glazer and D. A. Bryant, *Arch. Microbiol.* **104**, 15 (1975).
16. G. D. Offner and R. F. Troxler, *J. Biol. Chem.* **258**, 9931 (1983).
17. J. G. Belasco, J. T. Beatty, C. W. Adams, A. von Gabain, S. N. Cohen, *Cell* **40**, 171 (1985).
18. S. E. Curtis and R. Haselkorn, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1835 (1983).
19. E. O. Hughes, P. R. Gorham, A. Zender, *Can. J. Microbiol.* **4**, 225 (1958).
20. R. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982).
21. R. Thayer, *Anal. Biochem.* **98**, 60 (1979).
22. A. M. Maxam and W. Gilbert, *Methods Enzymol.* **65**, 499 (1980).
23. J. Messing, *ibid.* **101**, 20 (1983).
24. J. Norrander, T. Kempe, J. Messing *Gene* **26**, 101 (1983).
25. D. B. Stern, T. P. Hodge, D. M. Lonsdale, *Plant Mol. Biol.* **3**, 355 (1984).
26. S. A. Krawetz and R. A. Anwar, *BioTechniques* **2**, 342 (1984).
27. A. R. Cashmore, in *Methods in Chloroplast Molecular Biology*, M. Edelman, R. B. Hallick, N.-H. Chua, Eds. (Elsevier, New York, 1982), pp. 387–392.
28. P. Thomas, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5201 (1980).

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A *Plasmodium falciparum* Antigen That Binds to Host Erythrocytes and Merozoites

Abstract. *Antigens that bind to erythrocytes were identified in the supernatant fluids of a cultured human malaria parasite* (Plasmodium falciparum). *A 175-kilodalton (175K) antigen bound only to erythrocytes susceptible to invasion. The 175K antigen from the Camp or the FCR-3 strain also bound to merozoites. However, the antigen did not bind to merozoites when merozoites and supernatant antigens were from different strains unless proteinase inhibitors were present. Moreover, erythrocytes coated with supernatant antigens from the Camp or FCR-3 strain were invaded normally by merozoites of the homologous strain but were partially resistant to invasion by merozoites of the heterologous strain. The 175K antigen may be a receptor acting as a "bridge" between erythrocytes and merozoites.*

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The human malaria parasite *Plasmodium falciparum* invades only human and a few nonhuman primate erythrocytes (*1*). The erythrocytes are invaded by the merozoite form of the parasite, and the process of invasion depends on the capability of merozoites to recognize ligands on the erythrocyte membrane (*2–4*). Identification of parasite receptors and their ligands is important for understanding the invasion process and for developing immunologic and chemotherapeutic weapons against the parasite.

Most studies of merozoite-erythrocyte interactions indicate that sialic acid and erythrocyte membrane glycophorins are important ligands for invasion (*3*). Using purified erythrocyte ligands as affinity substrates, investigators have tried to identify receptors on the parasite. Parasite antigens with molecular weights of 140K, 70K, and 35K were characterized by their binding to purified sialoglycoproteins of the erythrocyte membrane (*5*). However, the role of these antigens during invasion has not been determined. Parasite antigens of 155K and 130K bind to purified glycophorin A (*6*), but not to normal erythrocytes (*7*). Another 155K antigen is inserted into the erythrocyte membrane at the time of invasion (*8*, *9*), and specific antibodies purified from a human immune serum inhibit the invasion of erythrocytes by merozoites (*10*). However, these antibodies do not inhibit the attachment of merozoites to erythrocytes (*10*). Therefore, although the glycophorin-binding antigens and the antigen inserted into the membrane of ring-infected erythrocytes are probably involved in invasion, there is no evidence that they mediate the initial attachment of merozoites.

In this study we used intact erythrocytes as an affinity substrate to identify other parasite molecules that may be involved in initial attachment. This approach has the advantage of maintaining erythrocyte ligands in their natural environment and with their native configuration. Since the use of intact erythrocytes precludes the use of detergents, supernatant fluids of *P. falciparum* cultures were used as a source of antigens. This approach seemed feasible since many antigens, including the 155K antigens, are released into culture supernatants (*6*, *8*). Erythrocyte-binding antigens (EBA) were therefore investigated by incubation of culture supernatants with erythrocytes that were susceptible or resistant to invasion in order to determine the specificity of the binding.

Schizont-infected erythrocytes from synchronized cultures (*11*) were purified (*12*) and labeled with [^{3}H]isoleucine. Culture supernatants were collected after 4 hours. Erythrocytes were incubated with the labeled supernatants, washed, and lysed in Triton X-100. The EBA in this lysate were precipitated with serum from a Nigerian donor containing malarial antibodies and were visualized by fluorography after sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (*13*).

Four antigens with apparent molecular weights of 175K, 120K, 65K, and 46K from culture supernatants bound to human and *Aotus trivirgatus* erythrocytes, both of which are susceptible to invasion (lanes b, e, i, and j in Fig. 1). Similar results were obtained with two different parasite strains, Camp and FCR-3 (*14*). No parasite antigens were detected in control experiments performed with serum from uninfected humans. The EBA were also investigated with erythrocytes treated with various enzymes and erythrocytes naturally resistant to invasion. No EBA were observed with human erythrocytes rendered resistant to invasion by treatment with trypsin (lane k in Fig. 1) or neuraminidase (lane 1 in Fig. 1). Binding of the 175K and 65K antigens did not occur with human Tn erythrocytes (lanes d and g in Fig. 1). These erythrocytes, which resist invasion, lack sialic acid on the *O*-linked tetrasaccharides of the glycophorins, but have sialic acid on *N*-linked oligosaccharides and on glycolipids (*15*). Erythrocyte-binding antigens of 120K, 85K, and 75K were revealed with guinea pig or rabbit erythrocytes, and EBA of 80K, 65K, and 46K were revealed with rhesus monkey