scription patterns are confined to regions of the salivary chromosomes that will eventually form puffs (*6*). Neither of these effects has been observed in cytological preparations after PEMF induction. Other types of pulses have been shown to initiate the uncoiling of DNA (*10, 11*).

It is appropriate to consider whether these findings can be correlated with the results of clinical studies in which the skeletal system was exposed to PEMF's. If transcription is affected by the availability of Ca^{2+}, as was suggested by our preliminary findings (*12*), this may affect the mode of SP and PT action both molecularly and clinically. Pulse trains are used clinically to elevate cellular calcium and to trigger calcification of fibrocartilage in disunited fractures (*3*). Single pulses, on the other hand, lower cellular calcium in chondrocytes (*13*) and stimulate bone accretion in patients with osteoporosis (*14*) and avascular necrosis (*4*). It is possible that PEMF's will find a variety of uses in other cases of cellular dysfunction. This study supports the hypothesis that PEMF's induce specific modifications in normal cell function. At least one effect can be directly related to transcriptional induction.

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Localization of a *Plasmodium* Surface Antigen Epitope by Tn5 Mutagenesis Mapping of a Recombinant cDNA Clone

Abstract. *A recombinant complementary DNA clone from* Plasmodium knowlesi *makes a β-lactamase fusion polypeptide in* Escherichia coli *that reacts with a monoclonal antibody to a* Plasmodium *surface antigen. An epitope of the surface antigen was localized by transposon Tn5 mutagenesis mapping of the complementary DNA clone. The Tn5 mutation having the farthest 5′ insert into the complementary DNA portion of the chimeric gene, giving the shortest truncated protein that maintained the ability to bind monoclonal antibody, defined the location of the epitope.*

We reported earlier the isolation of a complementary DNA (cDNA) clone (pEG81) from *Plasmodium knowlesi* (*1*). The cDNA clone makes a β-lactamase fusion polypeptide in *Escherichia coli* that reacts with a monoclonal antibody (2G3) to a circumsporozoite or CS protein (*2*). Since the cDNA insert into the pBR322 vector is only 340 base pairs (bp) long, the epitope against which antibody 2G3 reacts is limited to a region of the CS protein comprising about 110 amino acids. We now report further localization of the epitope to a 40–amino acid region

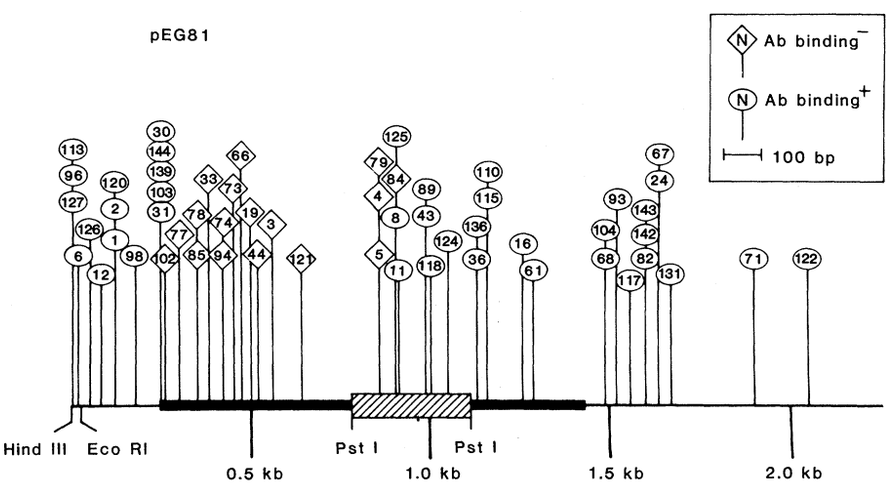

Fig. 1. A transposon Tn5 map of plasmid pEG81. The map shows the physical location of 57 independent Tn5 inserts (*13*). The insertion mutations were mapped with respect to the unique Hind III site of pEG81 by Hind III digest of purified plasmid DNA. Inserts on the right side of pEG81::Tn5-121 were digested with Pvu II plus Bam HI to demonstrate they were on the proper (Ap^r gene) side of pEG81. The Tn5 hops into the cDNA insert were confirmed by Pst I digestion; the disappearance of the 340-bp restriction fragment generated from Pst I digestion of pEG81 was judged as being due to Tn5 insertion into this fragment. The circled numbers indicate inserts that had no effect on the ability of lysate from cells bearing this plasmid to bind 2G3; lysates from cells containing plasmids with inserts indicated by numbers enclosed in diamond shapes no longer bound antibody (also see Table 1).

of the CS protein by a method in which clone pEG81 was mutagenized with transposon Tn5 insertion mutations (*3*).

The transposon Tn5 confers kanamycin resistance (*4*) and shows very little specificity at its insertion site (*5*). A Tn5 insertion into a gene destroys the gene function by insertional inactivation and exerts a polar effect on genes within an operon distal to its insertion (*6*). The physical location determined by transposition mapping correlates with the actual location as determined by nucleotide sequence (*7*); in the *E. coli dnaG* gene the limits of the gene were defined to within 50 bp.

Escherichia coli K-12 strain HB101 (*8*) harboring plasmid pEG81 was infected with a λ phage containing a Tn5 insert (λ::Tn5) and plated on Luria (LB) plates supplemented with tetracycline and kanamycin to select for the tetracycline-resistant (Tc^r) plasmid and the kanamycin-resistant (Km^r) Tn5. The λ::Tn5 is unable to integrate into the *E. coli* genome because the phage attachment site is deleted, and it cannot replicate because of amber mutations of λ genes O and P. Hence kanamycin addition selects for transposition of Tn5 from λ::Tn5 to the chromosome of the infected cells or to the plasmid they contain. Plasmid DNA was then isolated from Tc^rKm^r transductants and used to transform HB101 competent cells. The transformed HB101 cells were plated on LB plates supplemented with tetracycline and kanamycin to specifically select for plasmids containing a Tn5 insert. After single colony purification, plasmid DNA was isolated according to a rapid plasmid isolation procedure (*9*), and the position of the Tn5 was determined by restriction enzyme analysis.

A Hind III digest of plasmids containing a Tn5 insert into pEG81 (pEG81::Tn5) was used to determine the distance of the Tn5 from the unique Hind III site in pEG81. Restriction endonuclease Hind III cuts twice (*10*) in Tn5 in the inverted repeat region 1195 bp (*11*) from the end of the transposon. A Hind III digestion of plasmid pEG81::Tn5 generates three restriction fragments: the 3400-bp internal Hind III restriction fragment (*10*) and two junction fragments whose sizes are dependent on the relative position of Tn5 in the plasmid. If 1195 bp are subtracted from the smallest restriction fragment, the absolute distance from the unique Hind III site is determined. This does not determine on which side of the Hind III site the Tn5 is located. However selection of Tc^r clones eliminates the isolation of Tn5 plasmids with insertions into the Tc^r gene of plasmid pBR322. Any Tn5 insertion within 1000 bp from the Hind III site must therefore be on the side of pBR322 conferring ampicillin resistance (Ap^r). Plasmids with inserts further than 1000 bp from the Hind III site were digested with Pvu II plus Bam HI to determine whether or not the 1600-bp restriction fragment was generated from such a digest of pEG81 or whether its electrophoretic mobility was changed as a result of insertion by Tn5. From the 144 independent insertion mutations isolated, 57 were physically mapped to a specific region (Fig. 1). A typical result from a gel pattern of a Hind III digest of ten different inserts is shown in Fig. 2.

Table 1. Immunoradiometric assays of lysates from cells harboring plasmids with different Tn5 inserts. The IRMA's were performed as described (*1*). The experimental values obtained refer to the amount of ^{125}I-labeled antibody (counts per minute) bound by lysates from cells harboring pEG81 with different Tn5 insertion mutations. Lysates from cells containing pEG81 were used for the positive control and lysates from cells containing pBR322 were used as the negative control. The insert number refers to the individual Tn5 insertion mutations whose physical locations are shown in Fig. 1.

Insert	Positive control	Experimental	Negative control	Insert	Positive control	Experimental	Negative control
113	(10,700)	9800	(460)	4	(8,200)	830	(530)
96	(10,700)	9800	(460)	5	(8,200)	270	(530)
127	(10,700)	8000	(460)	125	(10,700)	2800	(460)
6	(8,200)	8500	(530)	84	(10,700)	1800	(460)
126	(10,700)	8400	(460)	8	(8,200)	5100	(530)
12	(8,200)	8500	(530)	11	(8,200)	7700	(530)
120	(10,700)	9900	(460)	89	(10,700)	2900	(460)
2	(8,200)	7700	(530)	43	(8,200)	2800	(530)
1	(8,200)	9200	(530)	118	(10,700)	3700	(460)
98	(10,700)	9900	(460)	124	(10,700)	9700	(460)
30	(8,200)	8500	(530)	136	(3,850)	3730*	(270)
144	(5,200)	4900*	(280)	36	(8,200)	7800	(530)
139	(5,200)	4850*	(280)	110	(10,700)	10000	(460)
103	(10,700)	11200	(460)	115	(10,700)	9200	(460)
31	(8,200)	8000	(530)	16	(8,200)	8800	(530)
102	(10,700)	1000	(460)	61	(8,200)	9000	(530)
77	(8,200)	560	(530)	104	(10,700)	9600	(460)
78	(10,700)	570	(460)	68	(8,200)	8500	(530)
85	(10,700)	570	(460)	93	(10,700)	8500	(460)
33	(8,200)	760	(530)	117	(10,700)	8800	(460)
74	(8,200)	420	(530)	143	(5,200)	4100*	(280)
94	(10,700)	620	(460)	142	(3,850)	3380*	(270)
73	(8,200)	730	(530)	82	(10,700)	8800	(460)
66	(8,200)	750	(530)	67	(8,200)	8100	(530)
19	(8,200)	1100	(530)	24	(8,200)	9800	(530)
44	(8,200)	550	(530)	131	(5,200)	4950*	(280)
3	(8,200)	3200	(530)	71	(8,200)	8800	(530)
121	(10,700)	6700	(460)	122	(10,700)	7200	(560)
79	(10,700)	360	(460)				

* Monoclonal antibody 5H8 was utilized. Both 5H8 and 2G3 react with the same region of the CS protein (*14*).

Lysates from *E. coli* cells containing plasmid pEG81 showed binding of the monoclonal antibody 2G3 in an immunoradiometric assay (IRMA), whereas lysates from cells containing pBR322 did not bind 2G3 antibody above background levels (*1*). This was the phenotype measured to determine the effect of Tn5 insertions into pEG81. Lysates from cells harboring pEG81::Tn5 plasmids were independently checked for their ability to bind 2G3 in the IRMA. The IRMA we used (*12*) was a sandwich assay requiring the presence of at least two epitopes. A two-site IRMA was used because pEG81 encoded information for more than one epitope (*1*). Initially the lysates were treated with a cold monoclonal antibody immobilized in a solid phase to isolate the antigen. This was then treated with ^{125}I-labeled 2G3 monoclonal antibody, and the number of radioactive counts bound reflected the amount of antigen. Lysates made from *E. coli* cells harboring pEG81 or pBR322 plasmids alone were used as controls to determine the counts bound in the presence and absence of the epitope, respectively. The IRMA data obtained for the 57 independent Tn5 insertions shown in Fig. 1 are listed in Table 1.

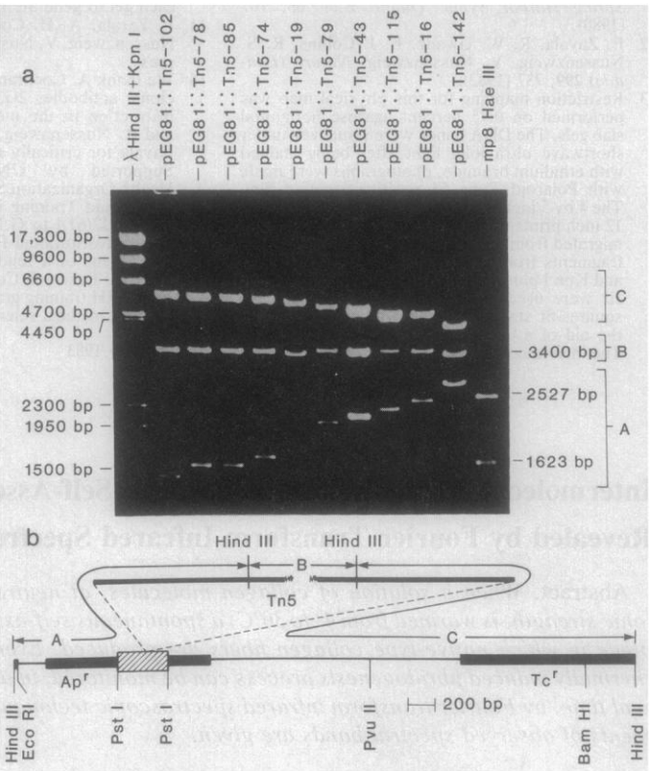

Fig. 2. Restriction patterns of different Tn5 inserts into pEG81. (a) A 0.75 percent agarose gel stained with ethidium bromide to make the DNA visible. The far left and right lanes are marker DNA's. On the left a Hind III plus Kpn I digest of phage λ generated the fragments shown; on the right a Hae III digest of phage M13mp8 DNA was used (the lower restriction fragments have run off this gel). Lanes 2 to 11 are ten different Tn5 inserts, each digested with Hind III. The inserts from left to right are ones that map farther and farther away from the unique Hind III site of pEG81. (b) A map demonstrating how the gel pattern in (a) is generated. The smaller fragments, labeled A, are those from the unique Hind III of pEG81 to the first Hind III in Tn5; B is the constant 3400-bp internal Hind III restriction fragment of Tn5; and C represents the fragment from the second Hind III in Tn5 back around to the unique Hind III of pEG81. See Fig. 1 for the location of these inserts with respect to the others on the map.

Insertion mutations in the region bound by the Hind III site (pEG81::Tn5-113) and the start of the Ap^r gene (pEG81::Tn5-31) have no effect on the number of counts bound in the IRMA. Any insert into the β-lactamase gene on the 5′ side of the cDNA insert (pEG81::Tn5-102 to pEG81::Tn5-121) destroys the ability to bind monoclonal antibody. This is presumably due to absence of antigen synthesis and is further evidence that it is the β-lactamase fusion polypeptide that reacts with 2G3. Interesting partial exceptions, pEG81::Tn5-3 and pEG81::Tn5-121, may be the result of initiation of RNA synthesis from a gratuitous Tn5 promoter (*6*) followed by translation initiated from the polyguanylate [poly(G)] sequence, which was generated during the original cDNA cloning [which used poly(C) · poly(G) tailing] to give antigen expression.

None of the Tn5 insertions on the 3′ side of the cDNA insert (pEG81::Tn5-136 to pEG81::Tn5-122) affect the ability of the lysate to bind 2G3. Transposon insertions into the cDNA portion of the fused ampicillinase gene, however, gave varying results. The physical location of pEG81::Tn5-8 and pEG81::Tn5-84 could not be further differentiated by gel patterns of Hind III–cleaved plasmid DNA; yet they bound labeled antibody with counts closer in value to the positive and negative controls, respectively. This therefore appears to be the 3′ boundary and the minimum amount of information needed to encode bivalent antigen. The Tn5 transpositions into the cDNA portion on the 5′ side of pEG81::Tn5-84 all give negative values for antigen production as judged by the small number of counts bound. Thus the bivalent epitope for 2G3 binding is delimited by the information encoded in an approximately 120 bp region of DNA, which corresponds to 40 amino acids. Since a sandwich IRMA was used (*12*), at least two epitopes are presumably contained within this region.

Localization of the epitope of the CS protein by transposition mapping suggests that only a small portion of the protein molecule, less than 40 amino acids, is required for IRMA reactivity. This in turn suggests that peptide synthesis, in conjunction with information gained from nucleotide sequence data, may be used to construct a synthetic epitope.

This method of in vivo mutagenesis of cDNA clones in *E. coli* by transposition mapping may have general applicability in defining eukaryotic phenotypes. The method can also be used to define the immunoreactive and enzymatic regions of the many eukaryotic genes that are already cloned and expressed in *E. coli* as β-lactamase or β-glactosidase fusion protein. Assays that depend on detection of a single epitope would be better suited for this purpose than the sandwich assay we used.

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Intermolecular Interactions in Collagen Self-Assembly as Revealed by Fourier Transform Infrared Spectroscopy

Abstract. *When a solution of collagen molecules, at neutral* p*H and moderate ionic strength, is warmed from 4° to 30°C, a spontaneous self-assembly process takes place in which native-type collagen fibers are produced. Events occurring during thermally induced fibrillogenesis process can be monitored, in aqueous media and in real time, by Fourier transform infrared spectroscopic techniques. Tentative assignments of observed spectral bands are given.*

Although there have been many studies of the fibrillogenesis reaction in vitro (*1, 2*), neither the mechanism of interaction nor the mode of initial assembly has been explained. The groups currently investigating this problem have taken different positions (*3–11*) with regard to the assembly mechanism. This report describes our studies of the collagen self-assembly process with a Fourier transform infrared spectrometer equipped for thermal-jump experiments.

The thermally induced self-assembly reaction involves two clearly distinguishable phases. First, a lag period occurs during which neither the solution turbidity nor the viscosity appears to change (*3, 4*), but during which some alteration in the solution properties must occur. A growth phase follows in which the turbidity increases rapidly and distinct fibrillar structures appear. Analysis of the nature of the interactions in the lag phase has been difficult because the methods used have perturbed the solution, have induced artifacts, or were insufficiently sensitive to detect the onset of interactions.

Helseth and Veis (*6*) suggested that two events might be of particular importance in fibrillogenesis: (i) a temperature-dependent intramolecular conformational change in the structure of the telopeptide regions, and (ii) a change in conformation of weak flexible regions within the triple helix, cooperatively assisted by intermolecular interactions with the telopeptides. Such interactions would be accompanied by changes in hydrogen bonding and bond strength.

Previous investigations of collagen structure by infrared spectroscopy were not directed to study of the mechanism of fibrillogenesis (*12–14*). The use of dry films meant that the studies began after collagen fibrils had already formed, so that no information on the collagen fibril aggregation process could be obtained. Therefore, these studies monitored the collagen-gelatin denaturation instead of the aggregation process. Moreover, the dry film studies could not address an important aspect of the physiological environment—the presence of buffered aqueous solutions. Dry collagen films have been studied in a highly humid or moist atmosphere (*15*). However, these data only demonstrate the absorption of water on collagen and not the effects of water solvation on collagen during fibrillogenesis. Raman studies have been carried out in both H_2O and D_2O solutions (*16*), but no attempt has been made to follow the fibrillogenesis process.

Fourier transform infrared (FTIR) spectroscopy, in which the hydrogen bond–related amide I, II, and III bands can be observed in H_2O, appeared to be an attractive nonperturbing means of studying both the lag and growth phases during self-assembly. The sensitivity of the method, which could be enhanced by accumulating scans over time intervals short compared to the kinetic processes being studied, was also promising. As described below, a system has been devised in which self-assembly can be followed directly in a thermostated infrared transmission cell.

Neutral salt–soluble collagen was extracted from lathyritic rat skin or tendon according to the protocol of Helseth and Veis (*6*). This collagen is essentially monomeric in solution and has a minimal content of β and γ components after denaturation. The gelation system of Comper and Veis (*3*), with 0.0327*M* KH_2PO_4 at *p*H 7.03, was used. In the experiment described below, the rat skin collagen concentration was 2.1 mg/ml and the ionic strength was adjusted to 0.15 with NaCl. This relatively high collagen concentration was used in all studies to maximize the infrared intensities. Under these conditions the turbidimetric lag, as determined with a double-beam spectrophotometer at 430 nm, was 3 to 5 minutes. The temperature jump from 3° to 30°C required 8 to 11 minutes (2 to 3 minutes to go from 3° to 28°C, the remainder to equilibrate to 30°C).

In the FTIR studies described here, similar temperature jump experiments were conducted in a standard transmission infrared cell from Harrick Scientific Corporation (Ossining, New York). The transmission cell temperature was maintained with a copper cooling coil wrapped around the cell housing. A heat transfer paste (Ecotherm TC-4, Emerson and Cummins Inc., Canton, Massachusetts) was applied to the cooling coil, housing, and cell window to promote rapid heating of the cell. The metal cell support plate was replaced by a Teflon support of similar size to decrease conductive heat transfer losses. The thermal gelatin temperature (30°C) was a compromise designed (i) to extend the lag period to several minutes, but (ii) to hold the temperature difference between the cell and the interferometer compartment (35°C) to a minimum.

In this transmission cell a period of 7 to 10 minutes was required to raise the cell temperature from 3° to 30°C (4 to 5 minutes to go from 3° to 28°C, the remainder to equilibrate to 30°C), nearly the same time as required for the temperature jump studies in the ultraviolet spectrophotometer. Temperature measurements were made in the FTIR cell with a thermocouple placed in a well drilled into the window at its periphery. A thermal gradient across the window could lead to a difference of perhaps 1° to 2° between the recorded cell temperature and the actual collagen solution temperature. This recorded cell temperature also takes into account any possible heating effects from the infrared radiation.

The infrared spectra were obtained