and runoff from the dump while the established growth of weeds and brush over the inactive dumpsite eliminated any visible airborne transport of dusts laden with PCB's. Downwind from the dump, the odor of PCB's was always apparent during the growing season. Within this area (and others where dusts contaminated with PCB's were not apparent), the differential in the foliar accumulation of PCB's persisted among the species studied. In some instances, the quantitative increases exceeded two orders of magnitude (Table 3). These data show that a site may be classified for the accumulation of PCB's in terms of multiples of the background level (MBL) values. The MBL values for a site are characteristically more uniform than values of the foliar content of PCB's. The S.D. for MBL values at a site are below ± 25 percent.

These results led to the establishment of field plots along an easterly transect, with each plot containing the major forage crop species of the area and one or more continuous PCB air monitors. Preliminary data from the tested forage crops indicate that there is more than a hundredfold range of PCB accumulations in harvested portions of various crops. It is expected that these studies will provide guidelines for the selection of forage crops to keep them within the federal limit for PCB's of 0.2 part per million (*8*) in areas where atmospheric PCB concentrations are elevated. Although this research is being done to predict contamination of vegetation from various sources of vapor-phase PCB's, perhaps of broader significance is the potential use of PCB measurements of vegetation to monitor annual changes in atmospheric PCB's.

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Expression of *Treponema pallidum* Antigens in *Escherichia coli*

Abstract. Treponema pallidum *DNA was cloned in a bacteriophage. Clones were screened for expression of* Treponema pallidum *antigens by an in situ radioimmunoassay on nitrocellulose, with the use of subsequent reactions with syphilitic serum and radioiodinated* Staphylococcus aureus *protein A. One clone, which gave a strong signal, codes for at least seven antigens that react specifically with human antibodies to* Treponema pallidum.

Syphilis continues to cause significant morbidity throughout the world despite the availability of penicillin. Yet the failure to culture the organism, in or on artificial media, has placed a severe restriction on the study of *Treponema pallidum*. The organism can be propagated in the rabbit testis, but purification of *T. pallidum* in its motile, virulent form has not yet been achieved. Thus, purified treponemal antigens are not available for experimental studies in biology, pathogenesis, serodiagnosis, and immunity.

Recombinant DNA technology offers the potential for producing substantial quantities of specific purified treponemal antigens. We report here the cloning and direct expression of *T. pallidum* DNA in *Escherichia coli*.

DNA extracted from relatively purified, motile, virulent *T. pallidum* grown in rabbit testicles (*1*) was partially digest-

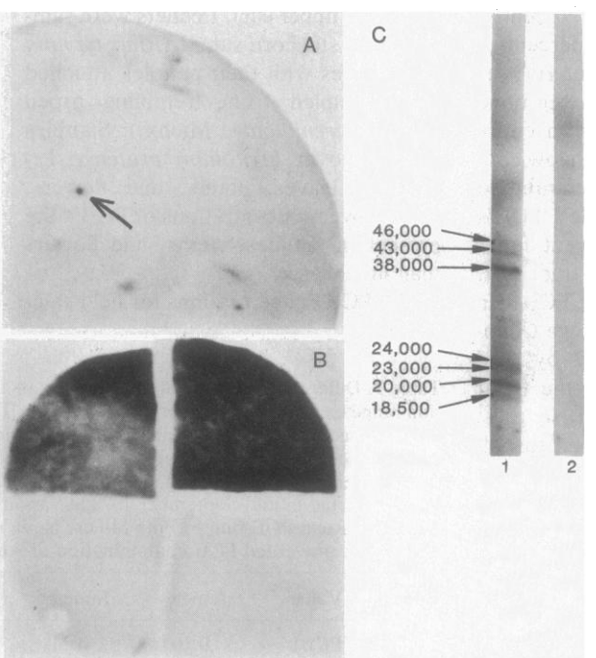

Fig. 1. (A) Autoradiogram of the primary screening of the *T. pallidum* gene bank. About 150 plaque-forming units of in vitro packaged recombinant Charon 30 were plated on a 150-mm petri dish of NZC agar with *E. coli* K802 (*3*). The soft top agar was made with agarose for greater strength. The plate was incubated and the grown phage was blotted to nitrocellulose. The blot was immunologically screened with human secondary syphilitic serum and ^{125}I-labeled protein A, and exposed to Kodak XAR-5. The plaque giving the strongest signal (arrow) was designated as clone Tp3a. (B) Serological specificity of Tp3a. Clone Tp3a was grown as almost confluent plaques and blotted onto a filter that was cut into quadrants. The upper quadrants were assayed with the serums from two patients with secondary syphilis, the lower quadrants with two serums from normal humans. (C) Molecular weight determination of *T. pallidum*–specific antigens expressed by Tp3a. Equivalent amounts of top agar containing plaques of Tp3a and Charon 30 were made up to final sample buffer concentrations of SDS and β-mercaptoethanol and boiled (*4*). The samples were placed on the gel in lanes 1 and 2, respectively, of a gradient (8 to 20 percent) polyacrylamide–SDS gel. After electrophoresis and transfer to nitrocellulose, the preparation was screened with the syphilitic serum used in the primary screening. The molecular weights were determined by comparison to ^{125}I-labeled standards ranging from 94,000 to 14,400 daltons (Pharmacia).

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ed with restriction endonuclease Sau 3AI, ligated to purified segments cleaved by Bam HI of coliphage Charon 30 (*2*), packaged in vitro (*3*), and used to infect *Escherichia coli* strain K802. The resulting plaques were screened for *T. pallidum* antigens by an in situ radioimmunoassay. Screening was done by a modification of the procedure of Towbin *et al.* (*4*). Nitrocellulose disks were placed over the phage plaques, and the disks were allowed to absorb protein for 10 to 30 minutes. Little protein was absorbed from the unlysed *E. coli* of the lawn. The nitrocellulose filters were then soaked for 10 minutes in 5 percent ovalbumin in 50 m*M* tris-HCl, *p*H 7.5, 150 m*M* NaCl, and 0.15 percent sodium azide (TSA–5 percent OA). The plaque blots were incubated overnight in either human secondary syphilitic serum or in normal human serum diluted 1:300 in TSA–1 percent OA, washed, exposed to ^{125}I-labeled *Staphylococcus aureus* protein A, and washed; autoradiographs were made as described (*4*). One plaque designated Tp3a, which gave a strong reaction with a secondary syphilitic serum (Fig. 1A), was chosen for further study. Phage from this plaque was diluted and replated on *E. coli* CSH 18. When screened with three different secondary syphilitic serums, all plaques showed radioactivity, whereas control plaques of the cloning vector, Charon 30, showed little or no reaction (data not shown). Secondary syphilitic serums reacted strongly with Tp3a plaques while serums from individuals without syphilis show no reaction with Tp3a plaques (Fig. 1B).

The polypeptide treponemal antigens of Tp3a were studied by electrophoretic transfer of total lysate proteins to nitrocellulose filters after sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (*4*) (Fig. 1C, lane 1). Tp3a encodes for at least seven clearly discernible polypeptides which have molecular weights of 46,000, 43,000, 38,000, 24,000, 23,000, 20,000, and 18,500 and react specifically with syphilitic serums. The molecular weights of these antigens correspond to those of antigenic proteins of *T. pallidum* (*5*). Identical or greater amounts of Charon 30 lysate proteins do not react with the syphilitic serums (Fig. 1C, lane 2). DNA isolated from Tp3a has been characterized by restriction endonuclease analysis (Fig. 2). Bacteriophage Tp3a contains a 16-kbp insert of *T. pallidum* DNA. The total molecular weight of the *T. pallidum* antigens is well within the coding capacity of this 16-kbp insert of cloned DNA.

There is a remote possibility that we have cloned a contaminating sequence of rabbit DNA because our original *T. pallidum* preparation was harvested from rabbit testicles. The treponemal origin of the Tp3a DNA was established on the basis of the antigenicity of its products with human syphilitic serums. Since secondary syphilitic serums do not react with protein blots of normal rabbit testicular tissue and normal rabbit serum (*5*), these serums were specific for detection of treponemal antigens. Further, two additional clones that produce Tp3a antigens were identified during a subsequent screening of 500 clones. Therefore, we have obtained three isolates having identical DNA sequences from a total of 750 clones, an indication that the genome size of the organism from which the DNA was cloned is the prokaryotic range of 10^8 to 10^9 daltons (*6*).

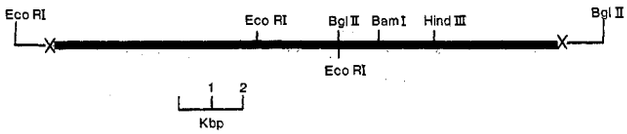

Fig. 2. Partial restriction endonuclease map of the *T. pallidum* DNA of clone Tp3a. The 16-kbp insert is circumscribed by Sau 3AI or Bam HI sites at the insert-vector junctions, indicated by X. The outer Eco RI site on the left is on the long Bam HI arm of Charon 30; the outermost Bgl II site on the right is on the short arm of Charon 30.

We expect that these and other cloned *T. pallidum* antigens will be the basis for new investigations of pathogenesis and immunity in experimental and human syphilis. In addition, antigens cloned in this manner should permit the development of treponemal tests exhibiting greater specificity than those presently in use. Hanff and Lovett have described the IgG (immunoglobulin G) antibody response of humans and rabbits to individual polypeptides of *T. pallidum* (*5*), and serums analyzed have included those obtained from patients and rabbits with immunity to reinfection. With unlimited amounts of cloned key treponemal antigens, it should be possible to duplicate through experimental vaccination the pattern of antibody to treponemal polypeptides seen in these immune states and test whether this antibody affords protection against the disease.

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Myoglobin Function in Exercising Skeletal Muscle

Abstract. *Short-term perfusion of the isolated dog gastrocnemius-plantaris muscle with hydrogen peroxide resulted in a decrease in steady-state muscle oxygen consumption and isometric tension generation. Hydrogen peroxide converted intracellular myoglobin to products incapable of combination with oxygen, but had no deleterious effect on neuromuscular transmission or on mitochondrial oxidative phosphorylation. It is concluded that functional intracellular myoglobin is important in maintaining oxygen consumption and tension generation in exercising skeletal muscle.*

Myoglobin is present in skeletal and cardiac muscle of mammals and birds in concentrations ranging from 0.05 to 5 mmole/kg and in the muscles and nerves of certain invertebrates (*1*). Notwithstanding its ubiquity, evidence is lacking with respect to its function. In the presence of an oxygen concentration gradient, myoglobin facilitates the transport of oxygen through solutions in physical systems (*1, 2*). DeKoning *et al.* (*3*) found that the flux of oxygen through sections of chicken gizzard was increased when myoglobin was present in its functional form. Wittenberg *et al.* (*4*) determined that chemical treatment of intracellular myoglobin that rendered it unreactive with oxygen resulted in a decrease in the steady-state respiration of pigeon breast muscle fiber bundles under conditions of hypoxia. In the isolated perfused heart, however, similar chemical treatments failed to produce this effect (*5, 6*). I report here that myoglobin is important

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