enzymes may be another example of divergent evolution where structural changes required for their distinctive functions have appeared only in the relatively compact catalytic area. Deletions in the DD-peptidase sequence could have produced a smaller β-lactamase molecule, but the overall structural scaffolding of the penicillin-binding proteins has been maintained in spite of primary sequence changes. It may also be hypothesized that the primary response of soil bacteria, like *Streptomyces* spp., to exposure to β-lactam compounds produced by other microorganisms was to develop an excretion mechanism permitting release of a membrane-bound PBP. Subsequently, improvement of this mode of detoxification resulted in the transformation of this water-soluble penicillin-binding enzyme into a penicillin-hydrolyzing enzyme.

Detailed modeling of the DD-peptidase structure to 1.6 Å resolution and extension of the β-lactamase structure with x-ray diffraction data to 2.0 Å may explain why β-lactam antibiotics are potent inhibitors of the target enzymes but are rapidly hydrolyzed substrates of the defensive β-lactamases.

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Tyr^{527} Is Phosphorylated in pp60$^{c\text{-}src}$: Implications for Regulation

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The Rous sarcoma virus oncogene product, pp60$^{v\text{-}src}$, transforms cultured fibroblasts but its corresponding proto-oncogene product, pp60$^{c\text{-}src}$, does not. Both proteins are known to be protein–tyrosine kinases. Published results suggest that the kinase activity of pp60$^{c\text{-}src}$ is inhibited relative to that of pp60$^{v\text{-}src}$, due perhaps to phosphorylation of a tyrosine in pp60$^{c\text{-}src}$ that is not phosphorylated in pp60$^{v\text{-}src}$. In this study, it was observed that the tyrosine phosphorylated in pp60$^{c\text{-}src}$ is Tyr^{527}, six residues from the COOH-terminus of the protein. The region of pp60$^{c\text{-}src}$ from residue 515 to the COOH-terminus, including Tyr^{527}, has been replaced with a different sequence in pp60$^{v\text{-}src}$. Thus, the increase in transforming ability and kinase activity that occurred in the genesis of pp60$^{v\text{-}src}$ may have resulted from the loss of a tyrosine involved in negative regulation.

THE CELLULAR PROTEIN PP60$^{c\text{-}src}$ IS the normal counterpart of the retroviral oncogene product, pp60$^{v\text{-}src}$ (*1*). Fibroblasts cannot be transformed by pp60$^{c\text{-}src}$ even when it is expressed at up to 15 times the normal level (*2–4*). In contrast, expression of pp60$^{v\text{-}src}$ at an equivalent level to endogenous pp60$^{c\text{-}src}$ induces transformation (*4, 5*). Both proteins are kinases that transfer phosphate to tyrosine residues in acceptor polypeptides, but when assayed in vitro with a variety of peptide substrates the specific activity of pp60$^{c\text{-}src}$ is about 2 to 10 percent of the activity of pp60$^{v\text{-}src}$ (*6, 7*). Comparison of their sequences shows that pp60$^{c\text{-}src}$ and pp60$^{v\text{-}src}$ differ in scattered point mutations and in their extreme COOH-termini: the last 19 residues of pp60$^{c\text{-}src}$ are replaced by an unrelated sequence of 12 residues in pp60$^{v\text{-}src}$ (*8–10*). Mutagenesis of c-*src* has shown that changes in either the body or the COOH-terminus of pp60$^{c\text{-}src}$ can cause transformation (*2*). Significantly, replacement of the unique COOH-terminal tail of pp60$^{c\text{-}src}$ by the pp60$^{v\text{-}src}$ sequence or by an arbitrary sequence is sufficient for transformation (*2, 3, 11*). Thus the COOH-terminal tail, in the context of the pp60$^{c\text{-}src}$ protein, apparently suppresses the transforming ability and protein kinase activity of pp60$^{c\text{-}src}$. How this occurs is unknown.

Phosphorylation of a tyrosine in pp60$^{c\text{-}src}$ appears to be important for regulation. In the cell, both pp60^{src}'s are phosphorylated at tyrosine and serine (*1*). Although the serines phosphorylated in pp60$^{c\text{-}src}$ and pp60$^{v\text{-}src}$ are the same, the major site of tyrosine phosphorylation is different (*12, 13*). The v-*src* protein is phosphorylated at Tyr^{416}, and pp60$^{c\text{-}src}$ is phosphorylated at an unknown tyrosine. In vitro, both pp60^{src}'s can autophosphorylate, principally at Tyr^{416} (*12*) and secondarily at one or more NH_2-terminal tyrosines (*14*). Thus, another protein kinase may phosphorylate the unknown tyrosine in pp60$^{c\text{-}src}$. Two pieces of evidence suggest that this phosphorylation may be inhibitory. Firstly, spontaneous mutations in c-*src* that activate its transforming ability encode proteins that are structurally very similar to pp60$^{c\text{-}src}$ but are not phosphorylated at the c-*src*–specific tyrosine (*6*). Secondly, activation of pp60$^{c\text{-}src}$ can occur after cell lysis because of the action of phosphatases that remove phosphate from the unique tyrosine (*15*). Here we report that pp60$^{c\text{-}src}$ is phosphorylated at Tyr^{527}, which lies in the COOH-terminal sequence that appears to suppress transformation. Phosphorylation of Tyr^{527} is probably critical for the regulation of pp60$^{c\text{-}src}$ kinase activity and transforming potential.

The tyrosine phosphorylation site in pp60$^{c\text{-}src}$ is known to lie in the COOH-terminal 26,000 daltons (*12*). For more precise mapping, we analyzed tryptic and chymotryptic peptides obtained from $^{32}P_i$-labeled pp60$^{c\text{-}src}$. Trypsin-digestion of pp60$^{c\text{-}src}$ from $^{32}P_i$-labeled mouse cells that express high levels of chicken pp60$^{c\text{-}src}$

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[NIH(pMc*src*/focus)B cells (*16*)] released phosphopeptides that contain phoshorylated Ser[12], Ser[17], and Ser[48] (*13*) as well as two or three hydrophobic peptides phosphorylated at tyrosine (Fig. 1a, peptides 6a and 6b). The phosphotyrosine-containing peptides were recovered in lower yield than expected from phosphoamino acid analysis or from partial digestion with *Staphylococcus aureus* V8 protease (*12*), perhaps because trypsin cleaved inefficiently at one or more uncharacteristic sites (*17*).

Incubation of peptides 6a and 6b with chymotrypsin (CT) yielded the same product (Fig. 1b, peptide A). Primary CT digestion of chicken pp60$^{c\text{-}src}$ derived either from NIH(pMc*src*/focus)B cells or chicken embryo cells also released peptide A and several minor peptides (Fig. 1, c and d). Peptide A contained phosphotyrosine (Fig. 2b, sample 1) while three minor peptides (B, C, and D) contained phosphoserine (*18*). Peptide A was unaltered by incubation with *Pseudomonas fragi* protease (cleaves before aspartic or cysteic acid), V8 protease (specific for glutamic acid not followed by proline), or trypsin, but was cleaved by a proline-specific endopeptidase (PE) (*19*).

By means of the amino acid sequence of chicken pp60$^{c\text{-}src}$ (*8*) and the cleavage specificity of CT (after phenylalanine, tryptophan, and tyrosine, but not after phosphotyrosine or before proline), we predicted the properties of all possible phosphotyrosine-containing CT peptides in the COOH-terminal 26,000 daltons of pp60$^{c\text{-}src}$. The observed properties of peptide A suggested that phosphorylation occurs at Tyr[527]. CT cleavage after either Tyr[519] or Phe[520] would then produce a phosphopeptide containing residues 520 to 533 or 521 to 533 (Fig. 3). This peptide would be cleaved by PE but not by *P. fragi* protease or trypsin, and may resist V8 protease owing to the proximity of Glu[531] to the COOH-terminus. Therefore, we synthesized a peptide (termed c*src*519-533) with the sequence predicted for the COOH-terminal 14 residues of pp60$^{c\text{-}src}$, containing both likely CT cleavage sites (Fig. 3). Antiserum to c*src*519-533 specifically immunoprecipitated peptide A, but not peptides B, C, or D, from a CT digest of $^{32}P_i$-labeled pp60$^{c\text{-}src}$ (*20*), providing strong evidence that peptide A contained sequences in c*src*519-533. We therefore directly compared peptide A with CT-digested c*src*519-533 that had been phosphorylated in vitro.

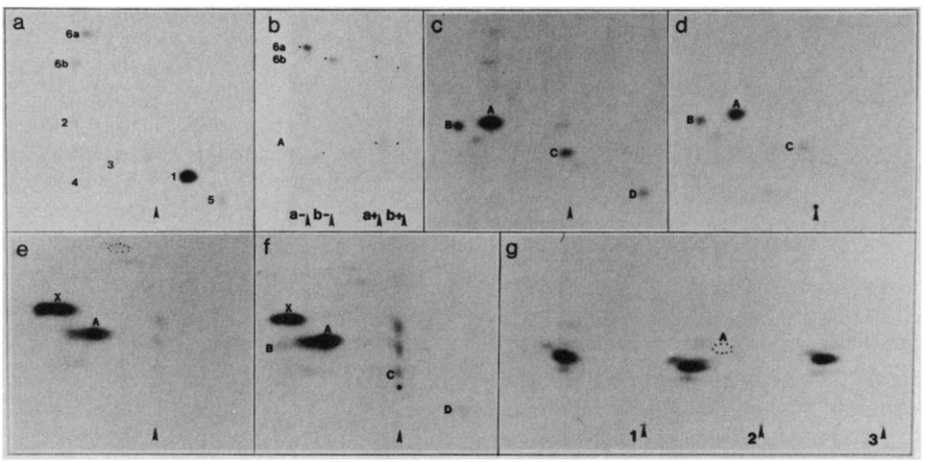

Fig. 1. Peptide maps of phosphorylated pp60$^{c\text{-}src}$ and of c*src*519-533. (a) Two-dimensional separation of tryptic phosphopeptides of pp60$^{c\text{-}src}$ from NIH(pMc*src*/focus)B cells. In (a) and (c–f), each digest (200 count/min by Cerenkov) was analyzed by electrophoresis at *p*H 8.9 (1.2 kV for 20 minutes) and chromatography (*27*), and exposures were for 4 days with a screen at −70°C. (b) One-dimensional chromatography of phosphotyrosine-containing tryptic peptides 6a (20 count/min) and 6b (10 count/min) from pp60$^{c\text{-}src}$, before (−) and after (+) digestion with CT (exposure for 10 days). (c–f) Two-dimensional separations of CT phosphopeptides derived from: (c) pp60$^{c\text{-}src}$ from NIH(pMc*src*/focus)B cells; (d) pp60$^{c\text{-}src}$ from chicken embryo cells; (e) in vitro phosphorylated c*src*519-533 (dots, approximate position of undigested phosphorylated c*src*519-533); (f) mixture of samples analyzed in (c) and (e). In (g) is shown the PE-digestion of phosphopeptide A that had been derived by CT digestion of: sample 1, $^{32}P_i$-labeled pp60$^{c\text{-}src}$ (50 count/min); sample 2, in vitro phosphorylated c*src*519-533 (50 count/min); sample 3, mixture of samples 1 and 2 (each at 25 count/min). The three samples were analyzed on the same thin-layer plate by electrophoresis at *p*H 8.9 (1.2 kV for 15 minutes) and chromatography (4 days exposure). Dots, approximate position of undigested sample 2. A, B, C, D, and X represent peptides discussed in the text. The pp60$^{c\text{-}src}$ was obtained from NIH(pMc*src*/focus)B cells (*16*) or chicken embryo cells that had been labeled with $^{32}P_i$ for 16 to 18 hours by immunoprecipitation with monoclonal antibody 327 (*28*), and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (*29*). Labeled pp60$^{c\text{-}src}$ was eluted, acid-precipitated with 10 μg of carrier protein, oxidized with HCOOOH, and digested with trypsin or with CT [two digestions for 4 hours at 37°C with 10 μg of protease (Cooper Biomedical)] (*30*). Phosphopeptides 6a, 6b, and A were purified by electrophoresis on cellulose thin layers at *p*H 8.9 followed by ascending chromatography, and were eluted with buffer, *p*H 1.9 (*31*). Phosphopeptides 6a and 6b, mixed with 5 μg of carrier c*src*519-533, were digested with 10 μg of CT at 37°C in 20 μl of 0.05*M* NH_4HCO_3 for 1 hour. Phosphopeptide A was similarly digested with 10 μg PE (Miles). Peptide c*src*519-533 was synthesized by the Merrifield procedure (*32*) and purified by gel filtration and cation exchange chromatography. Acid hydrolysis released the following amino acids (*33*): D, 1.0 mol (expected, 1); E + Q, 3.9 (3); F, 1.0 (1); G, 1.2 (1); L, 1.0 (1); P, 2.2 (2); S, 1.0 (1); T, 1.9 (2); Y, 1.5 (2). Peptides were phosphorylated by incubation at 30°C for 1 hour in 12-μl reactions containing 2 μ*M* [^{32}P]ATP (2500 Ci/mmol), 20 m*M* PIPES, *p*H 7.0, 10 m*M* $MnCl_2$, 0.1 m*M* Na_3VO_4, 0.5 m*M* dithiothreitol, and an immunoprecipitate of pp60$^{c\text{-}src}$ prepared from NIH(pMc*src*/focus)B cells with monoclonal antibody 327 (*28*). ^{32}P-labeled c*src*519-533 was purified by electrophoresis at *p*H 3.5 and eluted with *p*H 1.9 buffer (*31*). The peptide was digested with 10 μg of CT in 20 μl of 0.05*M* NH_4HCO_3 at 37°C for 4 hours.

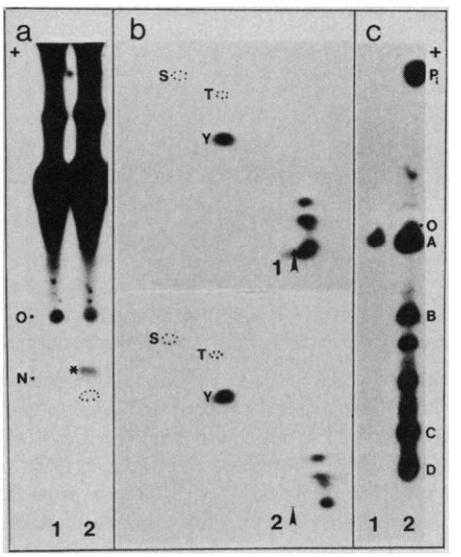

Fig. 2. Properties of synthetic peptide c*src*519-533. (a) Phosphorylation of peptide substrates by immunoprecipitates containing pp60$^{c\text{-}src}$: sample 1, no peptide added; sample 2, 5 m*M* c*src*519-533. Samples (1 μl) were analyzed by electrophoresis at *p*H 3.5, and exposed to film for 10 minutes in the presence of an intensifying screen. +, anode; O, sample origin; N, neutral dye (*N*-[2,4-dinitrophenyl]-ethanolamine); dots, ninhydrin-stained peptides; *, ^{32}P-labeled c*src*519-533. Free adenosine triphosphate moves toward the anode. (b) Products of partial acid hydrolysis of phosphopeptide A derived by CT-digestion of $^{32}P_i$-labeled pp60$^{c\text{-}src}$ (sample 1) and of ^{32}P-labeled c*src*519-533 (sample 2). The samples (50 count/min) were analyzed by electrophoresis at *p*H 1.9 (anode at left) followed by electrophoresis at *p*H 3.5 (anode at top) (*31*) and were exposed for 4 days at −70°C with a screen. S, phosphoserine; T, phosphothreonine; Y, phosphotyrosine. Partially hydrolyzed peptides run near the origin (arrowhead). (c) Products of CT-digestion of ^{32}P-labeled c*src*519-533 (sample 1, about 30 count/min) and of $^{32}P_i$-labeled pp60$^{c\text{-}src}$ (sample 2, 200 count/min), separated by electrophoresis at *p*H 1.9. Exposure was for 5 days at −70°C with a screen. O, origin; +, anode; P_i, orthophosphate; A–D, phosphopeptides. Neutral dye marker comigrated with A.

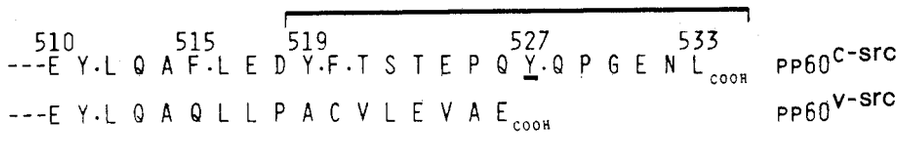

Fig. 3. COOH-terminal sequences of pp60$^{c\text{-}src}$ and pp60$^{v\text{-}src}$. The last 24 residues of pp60$^{c\text{-}src}$, predicted from the DNA sequences of chicken or human c-*src* genes (*8, 10*), are shown. The sequence of pp60$^{v\text{-}src}$, predicted from the sequences of Schmidt-Ruppin or Prague v-*src* genes, diverges between position 514 and the COOH-terminus (*9*). Dots, likely sites of CT cleavage; underline, Tyr^{527}; overline, synthetic peptide sequence. CT will not cleave after a phosphorylated tyrosine, so if Tyr^{519} is phosphorylated in pp60$^{c\text{-}src}$, cleavage will yield a phosphorylated peptide containing residues 516–520. If Tyr^{527} is phosphorylated, the phosphopeptide contains residues 520 or 521 to 533. Single-letter abbreviations for amino acids are shown (*33*).

When c*src*519-533 was incubated with immunoprecipitates containing pp60$^{c\text{-}src}$, it was phosphorylated exclusively at tyrosine (Fig. 2a, sample 2; Fig. 2b, sample 2) (*21*). There were two CT digestion products of ^{32}P-labeled c*src*519-533, which were both neutral at *p*H 1.9 (Fig. 2c, sample 1) but were separable by electrophoresis at *p*H 8.9 or chromatography (peptides X and A, Fig. 1e). Peptide A from ^{32}P-labeled c*src*519-533 appeared identical with peptide A derived from $^{32}P_i$-labeled pp60$^{c\text{-}src}$, when compared by electrophoresis at *p*H 1.9 (Fig. 2c) or by chromatography after electrophoresis at *p*H 8.9 (Fig. 1, e and f). Peptides A from both sources were resistant to V8 protease but were sensitive to PE, yielding the same major and minor products (Fig. 1g). Partial acid hydrolysis released the same partial cleavage products (Fig. 2b).

Since the synthetic peptide contains both Tyr^{519} and Tyr^{527}, either or both could be phosphorylated. The mobilities of peptide X are consistent with phosphorylation at Tyr^{519} and CT cleavage after Phe^{520} to yield phosphopeptide 519–520. Labeling of peptide X was sometimes not observed, consistent with the reported inefficiency of phosphorylation of NH_2-terminal tyrosine (*22*). It is clear that peptide A represents phosphorylation at Tyr^{527} for two reasons: a single cycle of Edman degradation of peptide A did not release the anilinothiazolinone of phosphotyrosine; and CT digestion of c*src*519-533 phosphorylated at Tyr^{519} and of pp60$^{c\text{-}src}$ phosphorylated at Tyr^{519} would yield different products (Fig. 3), which was not the case (Fig. 1).

These data show that the major site of tyrosine phosphorylation in chicken pp60$^{c\text{-}src}$ from fibroblasts is Tyr^{527}. Comparison of the labeling of Tyr^{527} (Fig. 1c, peptide A) with the labeling of Ser^{17} (Fig. 1a, peptide 1) suggests that Tyr^{527} contains as much or more phosphate. Indirect evidence implies that Ser^{17} is phosphorylated in most pp60$^{c\text{-}src}$ molecules (*13*), so the stoichiometry of phosphorylation of Tyr^{527} may approach 100 percent. High occupancy of this site may account for the absence of autophosphorylation at Tyr^{527}. Alternatively, Tyr^{527} may be a poor substrate for pp60$^{c\text{-}src}$ (*21*) or be inaccessible for autophosphorylation. Extensive Tyr^{527} phosphorylation is also consistent with its proposed role in suppressing pp60$^{c\text{-}src}$ kinase activity and transforming ability (*6, 15, 16*). This role contrasts with the presumed functions of COOH-terminal tyrosine phosphorylations in the EGF receptor (*23*). In this case, the tyrosines are not phosphorylated when the EGF receptor kinase is unstimulated, and autophosphorylation stimulates kinase activity, so the unphosphorylated sequence is presumed to be inhibitory.

Is Tyr^{527} phosphorylation regulated in the cell? Physiological activators of pp60$^{c\text{-}src}$ kinase activity are not known, but in polyoma virus–infected cells the pp60$^{c\text{-}src}$ complexed with middle T antigen has a greatly increased V_{max} for protein kinase activity (*14, 15, 24*). Recently we have found that this activated population of pp60$^{c\text{-}src}$ contains less than one-twentieth the phosphate at Tyr^{527} as free pp60$^{c\text{-}src}$ (*25*). We cannot conclusively attribute a primary role for Tyr^{527} phosphorylation in the regulation of pp60$^{c\text{-}src}$, however, since the activated pp60$^{c\text{-}src}$ contains phosphate at Tyr^{416}. Because autophosphorylation occurs at Tyr^{416}, phosphate at this residue may be a manifestation, rather than a cause, of increased kinase activity. The precise role of Tyr^{527} phosphorylation in pp60$^{c\text{-}src}$ function and the identities and regulation of the kinases and phosphatases involved are matters for further investigation. It is possible that these enzymes recognize distal regions of pp60$^{c\text{-}src}$ as well as the target tyrosine, accounting for the activation of pp60$^{c\text{-}src}$ as a transforming protein by NH_2-terminal mutations (*2, 6*).

Inhibition by tyrosine phosphorylation may not be unique to pp60$^{c\text{-}src}$. Two other cellular protein–tyrosine kinases, products of the *lsk*^{T}/*tck* and c-*fgr* genes, contain sequences identical to residues 526–529 of pp60$^{c\text{-}src}$ (*26*). Consistent with the idea that transforming protein–tyrosine kinases are not regulated by this mechanism, three closely-related oncogenes (v-*src*, v-*yes*, and v-*fgr*) diverge in sequence from c-*src* a few residues before Tyr^{527} (*1*). We predict that substitution of Tyr^{527} with another amino acid will result in constitutive activation of pp60$^{c\text{-}src}$, and generate a transforming protein.

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17. The charges and protease sensitivities of peptides 6a and 6b were not reconcilable with tryptic fragments predicted from the amino acid sequence (*8*).
18. J. A. Cooper, unpublished data. The three phosphorylated serines (Ser^{12}, Ser^{17}, and Ser^{48}) should lie in a single 51-residue CT peptide with a myristoylated NH_2-terminus. This product would probably not be detected in our system, and phosphopeptides B–D may represent infrequent sites of CT cleavage.
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asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

34. Since this report was submitted, A. P. Laudano and J. M. Buchanan (*Proc. Natl. Acad. Sci. U.S.A.*, in press) have reported that either Tyr^{511}, Tyr^{519}, or Tyr^{527} is phosphorylated in $pp60^{c\text{-}src}$.

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Specific DNA Probe for the Diagnosis of *Plasmodium falciparum* Malaria

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Malaria can be diagnosed either by direct microscopic examination of blood smears, which is time consuming and requires expertise, or by immunological techniques, which are effective but do not distinguish between past and present infections. In this study, a simple procedure was developed for spotting lysed blood from infected patients directly onto nitrocellulose paper and identifying the malaria species on the basis of hybridization of parasite DNA with a species-specific probe. A genomic DNA library of *Plasmodium falciparum* was screened to detect clones containing DNA sequences that are highly repeated within the parasite genome. Several such clones were further analyzed to identify those that hybridize specifically with *P. falciparum* DNA but not with DNA from humans, *P. vivax*, or *P. cynomolgi*. This technique appears to be sensitive enough to detect 10 picograms of purified *P. falciparum* DNA (equivalent to 100 parasites) and in field studies is able to detect approximately 40 parasites per microliter of blood.

APPROXIMATELY ONE THIRD OF THE world's population may be exposed to the risk of malaria infection (*1*). It is therefore not surprising that considerable effort has been directed toward the control, prophylaxis, and eventual eradication of this disease. Realization of these goals will ultimately depend on the availability of sensitive, simple, and inexpensive diagnostic means of detecting parasites in order to identify foci of infection and to evaluate the effect of various control programs.

Direct microscopic examination of blood smears continues to be the method of choice for diagnosing acute malaria. This method is both sensitive and specific since malaria can be differentiated from other potential infectious agents, and the various *Plasmodium* species can be readily distinguished. It is, however, time consuming to examine each slide, and a trained technician is required for accurate identification when low numbers of parasites are present. Immunodiagnostic procedures such as the enzyme-linked immunoabsorbant assay (ELISA) for detecting malaria antibodies are being developed and tested (*2*). These methods provide important information with regard to exposure to malaria, but, owing to the persistence of malaria antibodies after the disappearance of malaria parasites from the blood, such tests do not accurately discriminate between present and past infections. Immunodiagnostic methods for detecting antigen have also been developed (*3*). These are useful for detecting present infections, but antibody for malaria antigen from a past infection may interfere with this assay.

We report an alternative approach based on the use of cloned *P. falciparum* DNA for the specific detection of parasites in infected blood. This method detects current infections and should make it possible to measure

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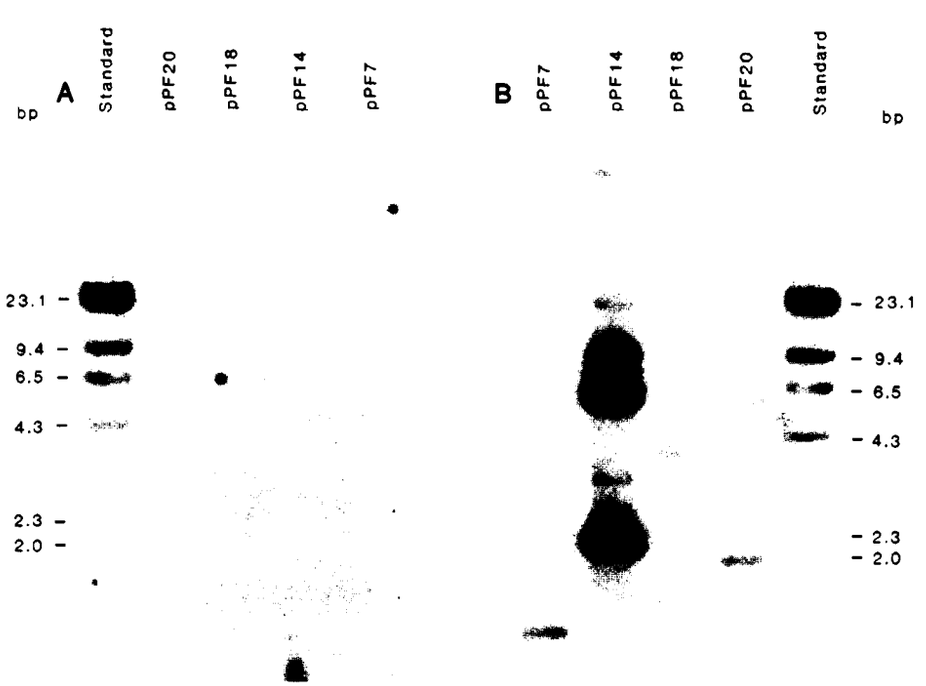

Fig. 1. Specific hybridization of recombinant plasmids with *P. falciparum* DNA but not with human DNA. Erythrocytes infected with the FCR3 strain of *P. falciparum* were maintained in vitro according to the method of Trager and Jensen (*12*). Infected erythrocytes were concentrated by gelatin sedimentation, lysed with detergent, treated with ribonuclease and proteinase K (Boeringer), and extracted with phenol-chloroform according to standard methods (*13*). *Plasmodium falciparum* DNA was then used to construct a genomic library. DNA was partially digested with Sau 3A (New England Biolabs) and was cloned into the Bam HI site of pBR322 before transformation of *Escherichia coli* strain HB 101. Transformed colonies were identified by sensitivity to tetracycline and were then screened by colony hybridization of duplicate nitrocellulose filters (0.45 μm, Schleicher & Schuell) using radiolabeled (nick-translated) DNA from either humans (placenta) or *P. falciparum*. Colonies were selected that hybridized strongly with *P. falciparum* DNA. Plasmid DNA was isolated from these colonies, digested, resolved, and transferred to nitrocellulose as described in the text (*14*). Filters were hybridized with either human (A) or *P. falciparum* (B) nick-translated DNA to confirm parasite specificity of cloned DNA. Numbers at the side are base pairs × 10^{-3}.