Essential Protein-Protein Interactions between Plasmodium falciparum Thymidylate Synthase and Dihydrofolate Reductase Domains*

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In Plasmodium falciparum, dihydrofolate reductase and thymidylate synthase activities are conferred by a single 70-kDa bifunctional polypeptide (DHFR-TS, dihydrofolate reductase-thymidylate synthase) which assembles into a functional 140-kDa homodimer. In mammals, the two enzymes are smaller distinct molecules encoded on different genes. A 27-kDa amino domain of malarial DHFR-TS is sufficient to provide DHFR activity, but the structural requirements for TS function have not been established. Although the 3′-end of DHFR-TS has high homology to TS sequences from other species, expression of this protein fragment failed to yield active TS enzyme, and it failed to complement TS- Escherichia coli. Unexpectedly, even partial 5′-deletion of full-length DHFR-TS gene abolished TS function on the 3′-end. Thus, it was hypothesized that the amino end of the bifunctional parasite protein plays an important role in TS function. When the 27-kDa amino domain (DHFR) was provided in trans, a previously inactive 40-kDa carboxyl-domain from malarial DHFR-TS regained its TS function. Physical characterization of the “split enzymes” revealed that the 27- and the 40-kDa fragments of DHFR-TS had reassembled into a 140-kDa hybrid complex. Thus, in malarial DHFR-TS, there are physical interactions between the DHFR domain and the TS domain, and these interactions are necessary to obtain a catalytically active TS. Interference with these essential protein-protein interactions could lead to new selective strategies to treat malaria resistant to traditional DHFR-TS inhibitors.

Malaria, caused by the protozoan parasite Plasmodium falciparum, affects about 300 million individuals and causes about 2 million death per year (1). Traditional antimalarial agents such as chloroquine are ineffective in many regions of the world due to drug resistance (2, 3). In addition, there is mounting evidence that highly drug-resistant parasite clones acquire resistance to new antimalarial agents at an enhanced rate (4, 5). Identification of host-parasite biochemical differences that can lead to selective chemotherapy is more important than ever before.

In malaria as well as many other cell types, chemotherapy targeted at dihydrofolate reductase (DHFR)1 and thymidylate synthase (TS) has proven to be highly effective (6, 7). Even partial inhibition of DHFR or TS can lead to DNA strand fragmentation and cell death (8–10). In malaria pharmacology, these two enzymes are of particular interest because traditional drugs such as pyrimethamine and cycloguanil are known to inhibit parasite DHFR-TS (11–13). In recent years, however, the effectiveness of these inhibitors has been compromised by malarial parasite strains expressing mutant forms of DHFR-TS (14–16). One obvious way to continue selective killing of malarial parasites is to identify new folate analogs directed at DHFR- or TS-active sites that are effective against drug-resistant parasites (17–20).

Beyond active site-directed strategies, there may be non-traditional opportunities to inhibit malarial DHFR-TS with selectivity. All protozoan parasites, including Plasmodium, have a single bifunctional protein that codes for both DHFR and TS activity (21–24). In sharp contrast, mammalian cells (as well as bacteriophage, bacteria, and yeast) have separate genes that code for small monofunctional DHFR and TS proteins (25, 26). This difference in organization of two well established drug targets represents a dramatic difference in host-parasite biochemistry. Yet, it has not been clear whether the bifunctional status of DHFR-TS can play a role in selective chemotherapy. First, all indications are that the active sites of DHFR and TS in these bifunctional proteins operate independently of each other. Inhibition of DHFR with methotrexate does not influence TS activity, and inhibition of TS function with 5-fluorodeoxyuridylic acid does not affect DHFR activity (27). The structure of Leishmania DHFR-TS suggests that the active sites of DHFR-TS are about 40 Å apart (28). Designing a “double-barrel,” parasite-specific bifunctional DHFR-TS inhibitor is not a practical goal at present. Second, kinetic measurements suggest that Leishmania and Toxoplasma TS may channel as many as 8 out of 10 dihydrofolate (DHF) molecules directly from the TS site to the DHFR site (27, 29, 30). Indeed, the Leishmania DHFR-TS structure indicates that this direct channeling may be attributed to an electropotential gradient in the bifunctional protein that leads from the TS folate-binding site to the DHFR folate-binding site (28, 29). However, it is highly unlikely that blocking such channeling will lead to selective antimalarial chemotherapy since malarial DHFR-TS active sites do not have any trouble delivering or accepting substrates through bulk solvent in vivo (31, 32) or in vitro (33).

In this study, we demonstrate that species-specific interactions between malarial DHFR domain and TS domain are essential for functioning of these enzymes. Inhibition of such protein-protein interactions may offer a powerful new strategy.

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† The abbreviations used are: DHFR, dihydrofolate reductase; TS, thymidylate synthase.
for selective chemotherapy against malaria and possibly other protozoan parasites.

EXPERIMENTAL PROCEDURES

Materials—Trimethoprim, ampicillin, tetracycline, leucine, proline, thymidine, 2′-deoxyuridylate, 5-fluoro-2′-deoxyuridylate, dihydrofolate, and methotrexate-agarose were from Sigma. Oligonucleotides were synthesized by Operon (Alameda, CA). Escherichia coli strains BL21, BL21(DE3), and a DE3 lysozymogen kit were from Novagen (Madison, WI). E. coli strain Rue10 (thyA) was from Chen-Chen Yan (Agouron Pharmaceuticals).

Plasmids—Plasmid pK2K32.2 with a P. falciparum DHFR-TS coding sequence of clone K1 was obtained from John Hyde (University of Manchester) and maintained as described previously (34). Plasmids pET23d(+) was from novagen (Madison, WI). This plasmid was cut with NcoI and HindIII and modified to express different domains of P. falciparum DHFR-TS (Fig. 1). pET-DHFR was designed to express amino acids 1–230 of the P. falciparum DHFR-TS coding region (32, 34); pET-TS31 expressed amino acids 325–607 of the the P. falciparum DHFR-TS coding region; pET-TS40 coded for amino acids 249–607 of the P. falciparum DHFR-TS coding region, and pET-DHFR-TS contained the full-length coding region (Fig. 1).

Antigen: Malarial DHFR Domain—The antigen, malarial DHFR domain, was expressed and purified on a methotrexate-affinity column as described previously (32). Rabbit polyclonal antibodies to this pure protein were raised by Josman Laboratories (Napa, CA). P. falciparum DHFR-specific antibody was purified by affinity chromatography using P. falciparum DHFR domain attached to Aminolink Plus from Pierce.

Genetic Complementation Assay for TS Function—The thymidylate synthase-deficient E. coli strain Rue10 was lysogesinated with ADE3 to provide T7 RNA polymerase function to this host (36). The host cells were maintained in LB medium in the presence of leucine (40 μg/ml), proline (200 μg/ml), and thymidine (100 μg/ml). When testing for TS function in pET plasmids bearing malarial DHFR-TS gene, or portions of this gene, 50 ng of plasmid was electroporated into about 4 ×109 cell E. coli strain Rue10(DE3) cells. The cells were spread on two sets of ampicillin agar plates, those with and those lacking thymidine.

Genetic Complementation Assay for Malarial DHFR Function—Appropriate pET plasmids were electroporated into BL21(DE3) cells as above. These cells were spread on two sets of ampicillin agar plates, those with trimethoprim (50 μg/ml) and those without. Bacterial DHFR is sensitive to trimethoprim but malarial DHFR is not (34).

Exonuclease Treatment of DNA Fragment Coding for Malarial DHFR-TS—The 5′-deletion experiment involved the following four steps prior to testing for TS function: (i) generation of a DNA strand with malarial DHFR-TS sequence that was susceptible to exonuclease on the 5′-end but not the 3′-end; (ii) digestion of the long fragment with exonuclease I at varying amounts of time to generate a series of DNA fragments of varying length; (iii) preparation of a recipient vector; the ligation products from each 2.5-min time point were maintained the full-length coding region (Fig. 1). These Rue10(DE3)plasmids were transformed into electrocompetent BL21(DE3) cells and plated on trimethoprim plates to measure DHFR function (see above). These Rue10(DE3)pET-DHFR were made electrocortent, transformed with pET-TS40, and selected for growth on medium lacking thymidine. Subsequently, it was found that double transformatants could also be obtained by first transforming cells with pET-TS40 (select on ampicillin plates) and then transforming them with pET-DHFR (selection on medium without thymidine).

Physical Characterization of P. falciparum TS40 + DHFR Split Products—E. coli Rue10(DE3) plasmids were ligated with pET-DHFR and pET-TS40, were freshly grown to an absorbance of 1.0 at 600 nm. The cells were collected by centrifugation and resuspended in 50 mM Tris-HCl, pH 8, 2 mM EDTA, 0.1% Triton X-100, and 0.1 mM glyoxime (final lysis volume was 1/10 of the original culture volume). The cells were incubated at 30 °C for 15 min, sonicated, and then stored in 1-ml aliquots at −20 °C.

RESULTS AND DISCUSSION

The Carboxyl End of Malarial DHFR-TS Is Insufficient for TS Function—The amino-terminal DHFR domain of malarial DHFR-TS can be expressed in functional form (32, 34, 37, 38), but all attempts to express the carboxyl end TS domain in a catalytically active form have been unsuccessful. A genetic system was used to assay for TS function from the malarial DHFR-TS gene. E. coli cells lacking TS function (strain Rue10) normally proliferate in the presence of thymidine but not in the absence of thymidine (39). Lysates prepared from Rue10 cells have DHFR enzyme activity but not TS activity (data not shown). When Rue10 cells are transformed with an expression plasmid coding for full-length malarial DHFR-TS gene, E. coli cells lacking TS function (strain Rue10) normally proliferate in the presence of thymidine but not in the absence of thymidine (39). Lysates prepared from Rue10 cells have DHFR enzyme activity but not TS activity (data not shown). When Rue10 cells are transformed with an expression plasmid coding for full-length malarial DHFR-TS gene, E. coli cells lacking TS function (strain Rue10) normally proliferate in the presence of thymidine but not in the absence of thymidine (39). Lysates prepared from Rue10 cells have DHFR enzyme activity but not TS activity (data not shown). When Rue10 cells are transformed with an expression plasmid coding for full-length malarial DHFR-TS gene, E. coli cells lacking TS function (strain Rue10) normally proliferate in the presence of thymidine but not in the absence of thymidine (39). Lysates prepared from Rue10 cells have DHFR enzyme activity but not TS activity (data not shown). When Rue10 cells are transformed with an expression plasmid coding for full-length malarial DHFR-TS gene, E. coli cells lacking TS function (strain Rue10) normally proliferate in the presence of thymidine but not in the absence of thymidine (39). Lysates prepared from Rue10 cells have DHFR enzyme activity but not TS activity (data not shown). When Rue10 cells are transformed with an expression plasmid coding for full-length malarial DHFR-TS gene, E. coli cells lacking TS function (strain Rue10) normally proliferate in the presence of thymidine but not in the absence of thymidine (39). Lysates prepared from Rue10 cells have DHFR enzyme activity but not TS activity (data not shown). When Rue10 cells are transformed with an expression plasmid coding for full-length malarial DHFR-TS gene, E. coli cells lacking TS function (strain Rue10) normally proliferate in the presence of thymidine but not in the absence of thymidine (39). Lysates prepared from Rue10 cells have DHFR enzyme activity but not TS activity (data not shown). When Rue10 cells are transformed with an expression plasmid coding for full-length malarial DHFR-TS gene, E. coli cells lacking TS function (strain Rue10) normally proliferate in the presence of thymidine but not in the absence of thymidine (39). Lysates prepared from Rue10 cells have DHFR enzyme activity but not TS activity (data not shown). When Rue10 cells are transformed with an expression plasmid coding for full-length malarial DHFR-TS gene, E. coli cells lacking TS function (strain Rue10) normally proliferate in the presence of thymidine but not in the absence of thymidine (39). Lysates prepared from Rue10 cells have DHFR enzyme activity but not TS activity (data not shown). When Rue10 cells are transformed with an expression plasmid coding for full-length malarial DHFR-TS gene, E. coli cells lacking TS function (strain Rue10) normally proliferate in the presence of thymidine but not in the absence of thymidine (39). Lysates prepared from Rue10 cells have DHFR enzyme activity but not TS activity (data not shown). When Rue10 cells are transformed with an expression plasmid coding for full-length malarial DHFR-TS gene, E. coli cells lacking TS function (strain Rue10) normally proliferate in the presence of thymidine but not in the absence of thymidine (39). Lysates prepared from Rue10 cells have DHFR enzyme activity but not TS activity (data not shown). When Rue10 cells are transformed with an expression plasmid coding for full-length malarial DHFR-TS gene, E. coli cells lacking TS function (strain Rue10) normally proliferate in the presence of thymidine but not in the absence of thymidine (39). Lysates prepared from Rue10 cells have DHFR enzyme activity but not TS activity (data not shown). When Rue10 cells are transformed with an expression plasmid coding for full-length malarial DHFR-TS gene, E. coli cells lacking TS function (strain Rue10) normally proliferate in the presence of thymidine but not in the absence of thymidine (39). Lysates prepared from Rue10 cells have DHFR enzyme activity but not TS activity (data not shown).
blunt-ended AUG start codon. The ligation mixes from each time point were transformed into E. coli Rue10(DE3) and scored for TS function (colonies growing without thymidine) and for malarial DHFR function (colonies growing in the presence of trimethoprim). The results showed the following: (i) DHFR and TS function were lost simultaneously (Fig. 2B); (ii) every colony that survived on plates without thymidine, when replica plated, had both malarial DHFR function and TS function (data not shown); and (iii) DNA sequencing of inserts from every TS E. coli showed an intact malarial DHFR-TS gene (data not shown). Control experiments confirmed that the repeated recovery of just the full-length DHFR-TS was not due to inability to digest the gene or high processivity of the exonuclease. In the early part of the 5'-digestion experiment, degradation of the malarial DHFR-TS gene could be visualized directly on agarose gel (data not shown). Intermediate sized fragments of appropriate length were observed when the DHFR-TS had been treated with exonuclease for 5–20 min (see schematic Fig. 2A). Additionally, selection of transformants in the presence of thymidine allowed recovery of inserts with incomplete DHFR-TS fragments (data not shown). These experiments were consistent with the hypothesis that the “carboxyl end TS domain” in malarial parasites was not adequate to obtain a functional TS enzyme. Furthermore, the exonuclease experiment suggested that the amino end of malarial DHFR-TS was necessary for TS function.

**DHFR Domain Can Activate TS Domain in Trans**—To test directly the contributions of malarial DHFR domain toward TS function, E. coli Rue10 transformed with the 40-kDa carboxyl TS domain were supplemented with the 27-kDa DHFR domain in trans and selected on medium lacking thymidine. The results showed that simultaneous transformation of these ThyA− E. coli with an expression vector coding for the 40-kDa TS domain and an expression vector coding for the amino end DHFR fully rescued the TS defect (Fig. 3). The two plasmids could be introduced in either order (data not shown). Polymer-
ase chain reaction analysis demonstrated that TS function was truly restored by genes acting in trans. There were two distinct plasmids in the rescued E. coli, and there was no evidence for a new bifunctional gene created through recombination between the plasmids (data not shown). Direct biochemical TS assay on cell lysates showed that, as expected, only the double transformed E. coli that proliferated without thymidine expressed active TS enzyme (data not shown). These experiments demonstrated unambiguously that the amino-terminal DHFR domain of malarial DHFR-TS was necessary for the carboxyl-terminal TS sequence to gain TS function. Since the TS domain could not be rescued by the DHFR domain (data not shown), the “joining region” must make significant contacts with the malarial DHFR domain. The requirement for DHFR domain was species-specific since endogenous E. coli DHFR could not activate malarial TS sequence expressed in the bacterial host.

Physical Associations within the Split Protein—There were two general ways in which the malarial DHFR domain could be activating TS function in the TS domain: (i) the DHFR domain could have acted as a transient chaperon during the folding of the TS sequence, or (ii) the DHFR domain could have formed a stable association with the carboxyl-terminal TS. To determine if there was stable association between the DHFR and TS domains of the split protein, the physical characteristics of the active proteins from the double transformed E. coli were studied. First, cell lysate from E. coli Rue10(DE3) pET-DHFR, pET-TS40, was subjected to gel filtration chromatography. TS activity and malarial DHFR emerged as a single superimposing peak (Fig. 4A). The size of the TS + DHFR complex was 140,000 daltons. This was consistent with the hypothesis that the DHFR and TS fragments had assembled into a tetramer made up of two 40-kDa TS units and two 27-kDa DHFR units, thereby reestablishing the natural relationship between the domains. All reasonable alternative structures are inconsistent with the gel filtration data. A free DHFR domain with a mass of 27 kDa was not seen on the gel filtration column. We also did not see a DHFR-free obligate TS dimer with a mass of 80 kDa.

Since the TS + DHFR split protein formed a complex stable enough to survive gel filtration chromatography, we chose to purify the complex to homogeneity and study its physical characteristics. Cell lysates from the double-transformed E. coli Rue10(DE3) were passed through a methotrexate affinity column, which is known to bind malarial DHFR domain (32). All unbound protein was eluted with 500 mM salt, and specifically bound protein was eluted with 4 mM dihydrofolate (Fig. 4B). Both the TS activity and the malarial DHFR activities eluted simultaneously in the 4 mM dihydrofolate fractions. The active fractions were concentrated and subjected to SDS-polyacrylamide gel electrophoresis (Fig. 4C). Coomassie Blue staining revealed two bands, one with a mass of 40 kDa and one with a mass of 27 kDa. Careful densitometry revealed that the 27- and the 40-kDa bands were present in a stoichiometric ratio of 1 to 0.97, after compensating for size differences. The larger 40-kDa band could be labeling with radioactive 5-fluoro-2’-deoxyuridyly-
interacts with residues of the TS domain and is necessary to form a catalytically active TS enzyme. Of course, it is also possible that, unlike the *Leishmania* bifunctional protein, the amino end of the malarial DHFR domain is necessary primarily to stabilize the DHFR tertiary structure and this, indirectly, influences correct folding and activity of the malarial TS domain.

Regardless of the mechanistic details underlying activation of the TS domain by the amino end of the DHFR domain, inhibition of these species-specific protein-protein interactions may offer a powerful new strategy for selective chemotherapy against malaria and possibly other protozoan parasites. Small molecular weight peptidomimetics that disrupt the interactions between the domains but fail to activate TS are expected to inhibit malarial TS with selectivity because human TS does not require “activation” by an accessory protein. There is precedence for drug development inspired by species-specific protein-protein interactions. For instance, virus-specific interactions between the two subunits of ribonucleotide reductase from herpes simplex virus led to selective peptidomimetic inhibitors that were effective at nanomolar concentrations (40). In addition, inhibition of protein-protein interactions is being considered in many systems including dimerization of HIV proteases, assembly of herpes simplex virus DNA polymerase subunits, interactions involving G-proteins and signal transduction machinery, and binding of adhesion molecules to surface proteins of platelets (41).

The present *E. coli* genetic assay for malarial TS function is expected to play a powerful role in future mutagenesis experiments that will help establish contact sites between the two malarial protein domains. In addition, the present genetic system will also serve well in high throughput screens to identify selective inhibitors of malarial TS function. Finally, the present “two-hybrid” genetic system provides a novel means for maintaining two plasmids indefinitely without any selectable drug marker.

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REFERENCES

1. Oaks, S. C. Jr., Mitchell, V. S., Pearson, G. W., and Pearson, C. C. J. (eds) (1991) *Malaria*, National Academy Press, Washington, D. C.
2. White, N. J. (1992) *J. Antimicrob. Chemother.* 29, 571–585
3. Young, T., Kirkman, L. A., Fujikawa, H., and Wellens, T. A. (1997) *Cell* 91, 593–601
4. Rathod, P. K., McErlane, T., and Lee, P.-C. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 9389–9393
5. White, N. J., and Olliaro, P. L. (1996) *Parasitol. Today* 12, 399–401
6. Hilleboe, B. L., and Blakely, R. B. (1986) *J. Biol. Chem.* 261, 2995–3001
7. Hitchens, G. H. (1980) *Clin. Pharmacol. Ther.* 1, 570–589
8. Houghton, P. J., Germain, G. S., Hazleton, B. J., Pennington, J. W., and Houghton, J. A. (1989) *Proc. Natl. Acad. Sci. U. S. A.* 86, 1377–1381
9. Inghram, H. A., Dickey, L., and Goulain, M. (1986) *Biochemistry* 25, 3225–3230
10. Yashima, A., Tanaka, S., Hiraoka, O., Koyama, H., Hirota, Y., Ayusawa, D., Sono, T., Garrett, C., and Wataya, Y. (1987) *J. Biol. Chem.* 262, 8235–8241
11. Hitchens, G. H. (1980) *Clin. Pharmacol. Ther.* 1, 570–589
12. Russell, P. B., and Hitchens, G. H. (1985) *J. Am. Chem. Soc.* 107, 5763–5770
13. Spinks, A., and Tottey, M. M. (1945) *Parasitol. Today* 12, 399–401
14. Foote, S. J., Galatis, D., and Cowman, A. F. (1990) *Proc. Natl. Acad. Sci. U. S. A.* 87, 3014–3017
15. Peterson, D. S., Milhous, W. K., and Wellens, T. E. (1990) *Proc. Natl. Acad. Sci. U. S. A.* 87, 3018–3022
16. Nair, N. M., Banerjee, S., and Sibley, C. H., Plowe, C. V., Winstanley, P. A., and Watkins, W. M. (1998) *Antimicrob. Agents Chemother.* 42, 164–169
17. Canfield, C. J., Milhous, W. K., Ager, A. L., Rossan, R. N., Sweeney, T. R., Lewis, N. J., and Jacobs, D. P. (1993) *Am. J. Trop. Med. Hyg.* 49, 121–126
18. Winstanley, P. A., Mberu, E. K., Swai, A. T., Karski, A. M., Watkins, W. M. (1995) *Antimicrob. Agents Chemother.* 39, 948–952
19. Hekmat-Nejad, M., and Rathod, P. K. (1997) *Exp. Parasitol.* 87, 222–228
20. Rathod, P. K., and Reshmi, S. (1994) *Antimicrob. Agents Chemother.* 38, 476–480
21. Bir, D. J., Li, W., Hori, T., and Insulburg, J. (1987) *Proc. Natl. Acad. Sci. U. S. A.* 84, 8360–8364
22. Beverley, S. M., Ellenberg, T. E., and Cordingly, J. S. (1986) *Proc. Natl. Acad. Sci. U. S. A.* 83, 571–585
23. McGee, R. S., and Olliaro, P. L. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 94, 9389–9393
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23. Chen, G., and Zolg, J. W. (1987) Mol. Pharmacol. 32, 723–730
24. Garrett, C. E., Coderro, J. A., Meek, T. D., Garvey, E. P., Claman, D., Beverly, S. M., and Santi, D. V. (1984) Mol. Biochem. Parasitol. 11, 257–265
25. Huennekans, F. M. (1996) Protein Sci. 5, 1201–1208
26. Carreras, C. W., and Santi, D. V. (1995) Annu. Rev. Biochem. 64, 721–762
27. Meek, T. D., Garvey, E. P., and Santi, D. V. (1985) Biochemistry 24, 678–686
28. Knighton, R. R., Kan, C.-C., Howland, E., Janson, C. A., Hostomska, Z., Walsh, K. M., and Mathews, D. A. (1994) Nat. Struct. Biol. 1, 186–194
29. Elcock, A. H., Potter, M. J., Matthews, D. A., Knighton, D. R., and McCammon, J. A. (1996) J. Mol. Biol. 262, 370–374
30. Trujillo, M., Donald, R. G., Roos, D. S., Greene, P. J., and Santi, D. V. (1996) Biochemistry 35, 6366–6374
31. Hekmat-Nejad, M., and Rathod, P. K. (1996) Antimicrob. Agents Chemother. 40, 1628–1632
32. Hekmat-Nejad, M., Lee, P., and Rathod, P. K. (1997) Exp. Parasitol. 85, 363–365
33. Fidock, D. A., and Wellems, T. E. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 10931–10936
34. Hall, S. J., Sims, P. F. G., and Hyde, J. (1991) Mol. Biochem. Parasitol. 45, 317–330
35. Moffat, B. A., and Studier, F. W. (1986) J. Mol. Biol. 189, 113–130
36. Rosenberg, A. H., Lade, B. N., Chai, D., Lin, S., Dunn, J. J., and Studier, F. W. (1987) Gene (Amst.) 56, 125–135
37. Sans, G., Morimatsu, K., and Horii, T. (1994) Mol. Biochem. Parasitol. 63, 265–273
38. Sirawaraporn, W., Prapunwattana, P., Sirawaraporn, R., Yuthavong, Y., and Santi, D. V. (1993) J. Biol. Chem. 268, 21637–21644
39. Belfort, M., and Pedersen-Lane, J. (1984) J. Bacteriol. 160, 371–378
40. Liuzzi, M., Deziel, R., Moss, N., Beaulieu, P., Bousquet, C., Chafouleas, J. G., Garneau, M., Jaramillo, J., Krogerud, R. L., Lagace, L., McCollum, R. S., Nawoot, S., and Guindon, Y. (1994) Nature 372, 695–698
41. Zutshi, R., Brickner, M., and Chmielewski, J. (1998) Curr. Opin. Chem. Biol. 2, 62–66