The Interaction of Escherichia coli Topoisomerase IV with DNA*

Hong Peng and Kenneth J. Marians

From the Molecular Biology Program, Memorial Sloan-Kettering Cancer Center and the Graduate Program in Molecular Biology, Cornell University Graduate School of Medical Sciences, New York, New York 10021

The two type II topoisomerases in Escherichia coli, DNA gyrase and topoisomerase (Topo) IV, share considerable amino acid sequence similarity, yet they have distinctive topoisomerization activities. Only DNA gyrase can supercoil relaxed DNA, whereas during orfC DNA replication in vitro, only Topo IV can support the final stages of replication, processing of the late intermediate and decatenation of the daughter molecules. In order to develop an understanding for the basis of the differential activities of these two enzymes, we have initiated a characterization of Topo IV binding to DNA. We find that unlike gyrase, Topo IV neither constrains DNA in a positive supercoil when it binds nor protects a 150-base pair region of DNA from digestion with micrococcal nuclease. Consistent with this, DNase I footprinting experiments showed that Topo IV protected a 34-base pair region roughly centered about the topoisomerase-induced cleavage site. In addition, Topo IV preferentially bound supercoiled rather than relaxed DNA. Thus, the DNA binding characteristics of Topo IV are more akin to those of the type II eukaryotic enzymes rather than those of its prokaryotic partner.

Four DNA topoisomerases (Topos) have been identified in Escherichia coli. Topo I (encoded by topA; Refs. 1 and 2) and Topo III (encoded by topB; Ref. 3) are type I enzymes. DNA gyrase and Topo IV are type II enzymes. Both gyrase and Topo IV are composed of two subunits: GyrA and GyrB (4, 5) and ParC and ParE (6), respectively. DNA sequence analysis shows that parC and parE have significant homology with gyrA and gyrB, respectively. Whereas these two topoisomerases share some common biochemical features, they can catalyze different reactions in vitro (7, 8) and appear to have different functions in vivo (9), although both enzymes are essential for cell survival.

In an ATP-dependent fashion, gyrase can supercoil relaxed DNA, create and decatenate DNA rings, knot and unknot circular DNA, and convert positive supercoils directly to negative ones. Gyrase will relax negatively supercoiled DNA in the absence of ATP (10). All Topo IV-catalyzed reactions require ATP. Topo IV will relax both positive and negative supercoils, knot and unknot DNA, and decatenate DNA rings (7, 11).

During DNA replication in vitro, only Topo IV is capable of supporting the terminal stages of replication, processing of the late intermediate and decatenation of the daughter molecules (8). Both gyrase and Topo IV can support nascent chain elongation during theta-type DNA replication in vitro (12). Genetic analysis has suggested that both Topo IV and gyrase are involved in chromosome decatenation (6, 13). This was supported by the study of Blishka and Cozzarelli (14) showing that gyrase was responsible for unlinking catenanes produced as a result of a recombination event. On the other hand, Adams et al. (9) showed that pBR322 replication catenanes accumulated at the nonpermissive temperature only in parC or parE strains, not in gyrA or gyrB strains.

In order to supercoil DNA, gyrase must be able to pass strands in one direction; otherwise only relaxation would occur. The mechanisms of gyrase-catalyzed reactions and the interaction of the enzyme with DNA has been studied extensively (15). When bound to DNA, gyrase constrains about 150 bp of DNA about itself in a positive toroidal supercoil (16-19). This is consistent with the results of both nuclease protection experiments (17) and DNase I footprinting experiments (18, 19). This ability of gyrase to order DNA locally with respect to the site of DNA cleavage during strand passage likely accounts for its supercoiling ability.

Both gyrase and Topo IV are targets for the quinolone and coumarin antibiotics (4, 5, 11, 20), yet in E. coli, resistance to these antibiotics arises only via mutation of the gyrase genes (21-24). Thus, although gyrase and Topo IV seem quite similar, their cellular functions are different. We have initiated an investigation into the mechanisms of the Topo IV topoisomerization activities in order to illuminate the structural basis for the differences in gyrase and Topo IV function. We find that unlike gyrase, Topo IV neither wraps DNA about itself nor distorts the path of the helix significantly on binding. Instead, the enzyme appears to bind a region of 34 bp centered about the cleavage site. Again, unlike gyrase, Topo IV prefers to bind supercoiled rather than relaxed DNA.

**Materials and Methods**

Preparation of Form I DNA—Form I pBSM13 DNA (80 μg, Stratagene) was treated with DNase I (0.13 μg, Pharmacia Biotech Inc.) for 30 min at 30°C in a reaction mixture (400 μl) containing 5 mM Tris-HCl (pH 7.6 at 30°C), 125 mM NaCl, 20 mM MgCl2, 100 μg/ml bovine serum albumin, and 1 μg/ml ethidium bromide. The reaction was stopped by the addition of EDTA to 50 mM. DNA was recovered by ethanol precipitation after phenol extraction and resuspended in 10 mM Tris-HCl (pH 7.6 at 4°C) and 1 mM EDTA.

Topoisomerase-induced Constraint of Supercoils of DNA—Reaction mixtures (30 μl) containing 50 mM Tris-HCl (pH 7.8 at 23°C), 10 mM MgCl2, 10 mM dithiothreitol, 26 μM NAD, 10 ng/ml trna, 25 μg/ml bovine serum albumin, form I pBSM13 DNA (0.4 μg), and the indicated amounts of either gyrase or Topo IV were incubated at 23°C for 20 min. E. coli DNA ligase (80 ng) was then added, and the reactions were continued for 1 h. EDTA, NaCl, SDS, and proteinase K were then added to 25 mM, 200 mM, 0.07%, and 3 μg/ml, respectively, and the incubation was continued for an additional 2 h. The DNA samples were then electrophoresed through 1.5% vertical agarose gels at 4 V/cm for 14 h in the presence of 13 μg/ml chloroquine phosphate using 50 mM Tris-HCl (pH 7.9 at 23°C), 40 mM NaOAc, and 1 mM EDTA as the electrophoresis buffer. The DNA was visualized by staining with ethidium bromide.

* These studies were supported by Grant GM 34558 from the National Institutes of Health and Grant NP 865 from the American Cancer Society. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: Topo, topoisomerase; bp, base pair; PCR, polymerase chain reaction.

2 H. Hiasa and K. J. Marians, unpublished data.
Topo IV Binding to DNA

Photonegatives of the gel were scanned using a Millipore Bi-Image Densitometer to determine the distribution of topoisomers.

Micrococcal Nuclease Protection—Reaction mixtures (50 μl) containing 50 mM Tris-HCl (pH 7.5 at 30°C), 20 mM MgCl₂, 1 mM CaCl₂, 2 mM dithiothreitol, 0.1 mM EDTA, 50 μg/ml bovine serum albumin, pBSM13 DNA labeled with ³²P by nick translation (60 fmol), and the indicated amount of either gyrase or Topo IV were incubated at 30°C for 20 min. The indicated amounts of micrococcal nuclease (Boehringer Mannheim) were then added, and the incubation was continued for an additional 10 min. EDTA, SDS, and proteinase K were then added to 50 mM, 0.2%, and 1 μg/ml, respectively, and the incubation was continued for 1 h. DNA products were then analyzed by electrophoresis through 7% polyacrylamide gels at 9 V/cm for 3.25 h using 89 mM Tris borate, 89 mM boric acid, and 1 mM EDTA as the electrophoresis buffer. Gels were dried onto Whatman 3 μ paper and visualized by autoradiography.

DNAse I Footprinting—The DNA substrate was prepared using the polymerase chain reaction (PCR) with plasmid pH101 (25) as the template and the oligonucleotide primers KanXho (5'-TCGAGGCCGCG-GATTATCTCAAC-3') and KanSma (5'-GGGATCCAGTCGTT-GAGTAAACCA-3'), one of which was ³²P-labeled using polynucleotide kinase. The resulting 276-bp DNA fragment, derived from sequences between the Xhol and Smal cleavage sites in the kanamycin resistance gene on pTH101, spans a strong Topo IV cleavage site. The PCR products were gel purified before use. DNAse I footprinting reaction mixtures (50 μl) containing the indicated amount of DNA fragment, 40 μM Tris-HCl (pH 7.6 at 30°C), 6 mM MgCl₂, 20 mM KCl, 2 mM dithiothreitol, and the indicated amounts of DNAse I were incubated at 30°C for 3 min. The indicated amounts of DNAse I (Pharmacia) were then added, and the incubation was continued for 30 s. A stop solution (20 μl) containing 100 mM EDTA and 125 μg/ml pBSM13 DNA was then added to terminate the reaction. The DNA was recovered by ethanol precipitation after extraction with a phenol-CHCl₃ mixture (1:1), resuspended in 6 μl of loading buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol ff) and electrophoresed through a 7% sequencing gel. The gel was dried onto Whatman 3 μ paper and visualized by autoradiography. Dideoxy DNA sequence ladders were prepared using the labeled KanXho and KanSma oligonucleotides as primers and pH101 DNA as template.

RESULTS

Topo IV Binds DNA Differently Than DNA Gyrase—DNA gyrase binds DNA in a very distinctive manner. The enzyme wraps roughly 150 bp of DNA about itself in a positive toroidal supercoil (15). Three different types of assays were used to detect this. These were (i) assessment of the alteration in the topology of relaxed DNA upon gyrase binding (16), (ii) assessment of the extent of DNA protected from micrococcal nuclease digestion as a result of gyrase binding (17), and (iii) DNAse I footprinting (17-19). We used these three assays to probe the mode of binding of Topo IV to DNA.

In order to assess whether Topo IV alters the path of the helix in a significant fashion when it is bound to DNA, Topo IV was bound to singly nicked form II DNA. The nick was sealed with DNA ligase, and the DNA was deproteinized and analyzed by electrophoresis through agarose gels. If binding of the enzyme constrains supercoils, they will become locked into the DNA upon covalent closure of the nick. They can then be observed easily by gel electrophoresis.

As reported previously for gyrase, binding of this enzyme to DNA resulted in the induction of supercoils after closure of the nick (Fig. 1, lanes 1–3). The photonegative of the stained gel was traced densitometrically to determine the shift in the position, compared with that in the absence of gyrase, of the center of the distribution of the topoisomers formed in the presence of gyrase. In this way, we could calculate that 0.5 superhelical turns were introduced to the DNA per bound gyrase tetramer. This is similar to the stoichiometry determined previously (16). Although not shown here, it has been determined previously that the superhelical turns induced by gyrase binding are positive (16).

The binding of Topo IV to the DNA resulted in a slight shift in the pattern of topoisomers toward a more positive distribution. This corresponded to the induction of 0.06 superhelical turns/Topo IV tetramer (Fig. 1, lanes 4–8). This was the case even at Topo IV to DNA ratios 4-fold higher than the ratio where gyrase-induced supercoiling was very obvious. Thus, it seemed unlikely that Topo IV was wrapping DNA about itself as gyrase does. Instead, it is possible that Topo IV unwinds duplex DNA somewhat upon binding.

To confirm that Topo IV was not wrapping DNA about itself, we determined the extent of DNA protected from micrococcal nuclease digestion by Topo IV binding. As established originally for nucleosomes (27), micrococcal nuclease will cut only in the spacer region between bound proteins. Thus, if a protein wraps DNA about itself, it should protect a significant region of the DNA from digestion by the nuclease. This is clearly observed for DNA gyrase. As reported previously (17), under protein to DNA ratios equivalent to one gyrase tetramer/200 bp of DNA, gyrase protected DNA in the size range of 110–160 bp from micrococcal nuclease digestion (Fig. 2A, lane 2). This is consistent with the ability of gyrase to wrap DNA about itself.

At 5-fold higher ratios of topoisomerase to DNA, the same pattern of protected DNA was evident for gyrase (Fig. 2B, lane 2), whereas Topo IV protected a wide range of DNA varying in size between the limit products of the micrococcal nuclease digestion to about 700 bp (Fig. 2B, lane 3). The wide size range of DNA protected by Topo IV under these conditions is most...
likely indicative of the binding to the DNA of multiple Topo IV tetramers close enough together to exclude access of micrococcal nuclease to the DNA.

In order to determine how large a region of DNA was bound by Topo IV, we performed DNase I footprinting. The substrate was a 276-bp DNA fragment made by PCR using plasmid pTH101 as a template. We had determined that this region of DNA had one major Topo IV cleavage site that could be observed in the absence of quinolones (data not shown). By labeling each primer separately, we were able to easily observe the Topo IV footprint on each DNA strand.

The results of the DNase I footprinting analysis (Fig. 3) showed that like all known type II topoisomerases, the Topo IV cleavage sites on the top (Fig. 3D) and the bottom (Fig. 3B) strands were staggered by 4 nt (Fig. 3C). Topo IV protected from DNase I digestion about 34 nt of DNA on each strand roughly centered about the cleavage site (Figs. 3, A–C). Because the cleavage site is staggered, this results in slightly asymmetric protection of the duplex from nuclease digestion. Thus, it seems that the mode of Topo IV binding to DNA is distinct from that of gyrase and is similar to that of the eukaryotic type II topoisomerases (28, 29).

Topo IV Binds Preferentially to Supercoiled DNA—The studies described in the previous section indicated that gyrase and Topo IV bound DNA differently. Gyrase binds preferentially to relaxed rather than supercoiled DNA (30), consistent with supercoiling being its primary function. Because Topo IV has no supercoiling activity, it seemed likely that the enzyme would bind supercoiled DNA preferentially. This was investigated using nitrocellulose filter binding assays. Topo IV binding to supercoiled (form I), relaxed (form II), and
linear (form III) pBR322 DNAs was compared (Fig. 4). The form I' DNA was prepared by treatment of form I DNA with E. coli Topo I. The resultant preparation contained no detectable form I DNA and about 5% form II (nicked) DNA. The form III DNA was prepared by digestion of form I DNA with the EcoRI restriction endonuclease. $K_D$ was calculated according to Riggs et al. (31).

Topo IV bound to form I, I', and III DNAs with $K_D$ values of 0.6 nM, 3.3 nM, and 9.3 nM, respectively. Because these DNAs were topological isomers, the different affinities of Topo IV for them can only be attributed to their different topological states. Thus, as predicted, unlike gyrase, Topo IV clearly bound supercoiled DNA preferentially to relaxed DNA. This was confirmed by a competition binding experiment.

Topo IV binding to form III 5'-[32P]pBSM13 DNA was competed by either unlabeled form I or form III pBSM13 DNA (Fig. 5A). Calculation of the amount of competitor needed to reduce binding to the [32P]-labeled DNA by 50% (Fig. 5, B and C) showed that an 18-fold higher molar excess of linear compared with supercoiled DNA was required. This was in good agreement with the nearly 16-fold difference in $K_D$ determined for binding of Topo IV to form I and III DNAs (Fig. 4).

Whereas it was clear that Topo IV bound supercoiled DNA better than relaxed DNA, it also seemed that of the two types of relaxed DNA used in the binding experiments, Topo IV bound form I' DNA roughly 3-fold better than form III DNA. Because the only difference between these two DNA forms is that the latter has ends and the former does not, we considered whether the difference in binding affinities could be accounted for as a result of Topo IV molecules sliding off the linear form. A similar explanation was raised to account for the reduced binding affinity of the Droso phila type II enzyme to form III DNA compared with form I DNA (32).

Experiments from Wang's lab (33, 34) suggest that the eukaryotic type II topoisomerases are possessed of an annular DNA binding site, and Sekiguchi and Shuman (35) have shown that the vaccinia type I enzyme binds DNA circumferentially. Thus, we prepared [35S]Topo IV and measured its binding to form I' and form III DNAs by gel filtration. At very low concentrations of Topo IV (<5 nM), we observed 50% more Topo IV bound to form I' compared with form III. However, this difference was lost at higher concentrations (data not shown). Thus, whereas sliding of Topo IV off of form III DNA may account for some of the observed binding differences, it cannot account for the full effect. It is of course possible that the EcoRI cleavage used to generate the form III DNA disrupted a high affinity binding site.

**DISCUSSION**

E. coli has two type II topoisomerases, DNA gyrase and the recently discovered Topo IV. Even though these enzymes share considerable amino acid sequence similarity (6), they support different reactions during DNA replication in vitro (8) and appear to behave distinctively in vivo (9).

Both enzymes can support nascent chain elongation during oriC DNA replication reconstituted in vitro with purified proteins (8, 12), although only Topo IV can support the terminal stages of replication, processing of the late intermediate and decatenation of the linked daughter molecules (8). Gyrase, but not Topo IV, has been implicated as the enzyme responsible for supporting chain elongation in vivo (8), although conclusions based on the effects on DNA replication of the quinolone antibiotics (36) must now be considered questionable because both gyrase (4, 5) and Topo IV (7, 11) are sensitive to these drugs.

Mutations that display a par phenotype can be mapped to...
both the gyrase (37, 38) and Topo IV genes (6, 39–41) and incompletely segregated nucleoids have been observed in gyrB mutant strains at the nonpermissive temperature (13). On the other hand, Adams et al. (9) demonstrated that catenated pBR322 daughter molecules arise at the nonpermissive temperature only in parC and parE strains, not in gyrA or gyrB strains.

In order to better appreciate the basis for the differential action of these two topoisomerases, we have investigated the interaction between Topo IV and DNA. Binding of gyrase to DNA is distinctive. The enzyme wraps roughly 150 bp of DNA about itself in a positive toroidal supercoil (15). It has been proposed that this ordering of the DNA across the surface of the DNA cleavage site facilitates the vectorial strand passage required for supercoiling (42). DNA bound to Topo IV in a manner more reflective of a eukaryotic type II topoisomerase than of gyrase.

Topo IV protected a small region of 34 bp from attack by DNase I when bound to DNA. Given that the Stokes radius of Topo IV is 65 Å (11), it is highly unlikely that the enzyme wraps DNA about itself. This is supported by the observation that the binding of Topo IV to DNA followed by the subsequent closure of the DNA into a ring did not result in the induction of positive supercoils, as was the case for gyrase (16). Thus, the size of the binding site of Topo IV on DNA is similar to that of the eukaryotic type II enzyme, which has an identical Stokes radius and protects 25–28 bp of DNA from nuclease digestion (28). In spite of the different mode of binding to DNA, Topo IV and gyrase binding sites appear to be dictated by similar sequence features because they overlap considerably (11). Perhaps the different modes of DNA binding accounts for the observed difference in substrate binding preference because they overlap considerably (11). Perhaps the different mode of binding to DNA, Topo IV and gyrase, protects 25–28 bp of DNA from nuclease digestion (28). In spite of the different mode of binding to DNA, Topo IV and gyrase binding sites appear to be dictated by similar sequence features because they overlap considerably (11). Perhaps the different modes of DNA binding accounts for the observed difference in the interaction between them and their role in the cell.

Acknowledgments—We thank David Valentine for the artwork.

REFERENCES
1. Trucksis, M., and Depew, R. E. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 2164–2168
2. Sternganz, R., DiNardo, S., Voelkel, K., Ashimura, Y., Hirota, Y., Becherer, K., Zumsteg, L., and Wang, J. C. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 2747–2751
3. DeGute, R. J., and Marrian, K. J. (1989) J. Biol. Chem. 264, 17924–17930
4. Gelert, M., O’Dea, M. H., Itoh, T., and Tomizawa, J. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 4474–4478
5. Gelert, M., Mizuchi, K., O’Dea, M. H., Itoh, T., and Tomizawa, J. I. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 4772–4776
6. Kato, J., Imanura, R., Niki, H., Hiraga, S., and Suzuki, H. (1990) Cell 63, 493–496
7. Kato, J., Nishimura, Y., Suzuki, H., and Ikeda, H. (1992) J. Biol. Chem. 267, 25676–25684
8. Peng, H., and Marrian, K. J. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 8571–8575
9. Adams, D. E., Sheehkinman, E. M., Zehnlewhite, E. L., Schmid, M. B., and Cozzarelli, N. R. (1992) Cell 71, 277–288
10. Gelert, M. (1981) Annu. Rev. Biochem. 50, 879–910
11. Peng, H., and Marrian, K. J. (1993) J. Biol. Chem. 268, 24481–24490
12. Hiasa, H., and Marrian, K. J. (1994) J. Biol. Chem. 269, 16371–16375
13. Steck, T. R., and Orilica, K. (1984) Cell 36, 1081–1088
14. Bliksa, J. B., and Cozzarelli, N. R. (1987) J. Mol. Biol. 194, 205–218
15. Reece, R. J., and Maxwell, A. (1991) Crit. Rev. Biochem. Mol. Biol. 26, 335–375
16. Liu, L. F., and Wang, J. C. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 2098–2102
17. Liu, L. F., and Wang, J. C. (1978) Cell 15, 979–984
18. Kirkegaard, K., and Wang, J. C. (1981) Cell 23, 721–729
19. Fisher, L. M., Mizuchi, K., O’Dea, M. H., Ohmori, H., and Gelert, M. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 4165–4169
20. Sugino, A., Peebles, C. L., Kreutzer, K. N., and Cozzarelli, N. R. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 4767–4771
21. Ryan, M. J. (1976) Biochemistry 15, 3769–3777
22. Gross, W. A., Deych, L. H., and Cook, T. M. (1966) J. Bacteriol. 92, 1068–1074
23. Staudenbauer, W. L. (1976) Eur. J. Biochem. 62, 491–497
24. Maxwell, A. (1991) J. Antimicrob. Chemother. 30, 409–414
25. Hill, T. M., Pelletier, A. J., Tiedennburg, M., and Kuempel, P. L. (1988) Cell 55, 459–466
26. Marrian, K. J., Soeller, W., and Zipurski, S. L. (1982) J. Biol. Chem. 257, 5656–5662
27. Noti, M. (1974) Nature 249, 251–252
28. Lee, M. P., Sander, M., and Hsiet, T. (1989) J. Biol. Chem. 264, 21779–21787
29. Thomsen, B., Bender, C., Lund, K., Andersen, A. H., Sorensen, B. S., and Westergaard, O. (1990) J. Mol. Biol. 215, 237–244
30. Higgins, N. P., and Cozzarelli, N. R. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 6833–6847
31. Riggs, A. D., Suzuki, H., and Bourgeois, S. (1970) J. Mol. Biol. 48, 67–83
32. Osheroff, N. (1986) J. Biol. Chem. 261, 9944–9950
33. Roca, J., Berger, J. M., and Wang, J. C. (1993) J. Biol. Chem. 268, 14250–14255
34. Roca, J., and Wang, J. C. (1992) Cell 71, 833–840
35. Sekiguchi, J., and Shimizu, S. (1994) J. Biol. Chem. 269, 31731–31734
36. Kreutzer, K. N., and Cozzarelli, N. R. (1970) J. Biol. Chem. 245, 424–435
37. Hussain, K., Elliott, E. J., and Salmond, G. P. (1987) Mol. Microbiol. 1, 259–273
38. Kato, J., Nishimura, Y., and Suzuki, H. (1989) Mol. & Gen. Genet. 217, 178–181
39. Kato, J., Nishimura, Y., Yamada, M., Suzuki, H., and Hirota, Y. (1988) J. Bacteriol. 170, 3967–3977
40. Schmid, M. B. (1990) J. Bacteriol. 172, 5416–5424
41. Luttinger, A. L., Springer, A. L., and Schmid, M. B. (1991) New Biol. 3, 687–697
42. Morrison, A., and Cozzarelli, N. R. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 1416–1420
43. Wasserman, S. A., White, J. H., and Cozzarelli, N. R. (1988) Nature 334, 468–450
44. Marrian, K. J. (1987) J. Biol. Chem. 262, 10362–10368
The Interaction of *Escherichia coli* Topoisomerase IV with DNA
Hong Peng and Kenneth J. Marians

*J. Biol. Chem.* 1995, 270:25286-25290.
doi: 10.1074/jbc.270.42.25286

Access the most updated version of this article at [http://www.jbc.org/content/270/42/25286](http://www.jbc.org/content/270/42/25286)

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/270/42/25286.full.html#ref-list-1) to choose from all of JBC's e-mail alerts

This article cites 44 references, 23 of which can be accessed free at [http://www.jbc.org/content/270/42/25286.full.html#ref-list-1](http://www.jbc.org/content/270/42/25286.full.html#ref-list-1)