Enhanced Processing of UVA-irradiated DNA by Human Topoisomerase II in Living Cells*[^S]

Received for publication, January 20, 2004, and in revised form, March 9, 2004.
Published, JBC Papers in Press, March 23, 2004.
DOI 10.1074/jbc.C400032200

Christian Mielke, Morten O. Christensen, Hans Ulrich Barthelmes, and Fritz Boege
From the Institute of Clinical Chemistry and Laboratory Diagnostics, Heinrich-Heine-University, Medical School, Moorenstrasse 5, D-40225 Düsseldorf, Germany

Solar UV light induces a variety of DNA lesions in the genome. Enhanced cleavage of base modifications by topoisomerase II has been demonstrated in vitro, but it is unclear what will arise from an interplay of these mechanisms in the genome of a living cell exposed to UV light. To address this question, we have subjected cells expressing biofluorescent topoisomerase IIα or IIβ to DNA base modifications inflicted by a UVA laser at 364 nm through a confocal microscope in a locally confined manner. At DNA sites thus irradiated, we observed rapid, long term (>90 min) accumulation of topoisomerase IIα and IIβ, which was accompanied by a decrease in mobility but not immobilization of the enzyme. The catalytic topoisomerase II inhibitor ICRF-187 prevented the effect when added to the cell culture before the UVA pulse but promoted it when added thereafter. Self-primed in situ extension with rhodamine-DUTP revealed massive DNA breakage at the UVA-exposed spot. Culturing the cells with ICRF-187 before UVA-exposure prevented such breaks. In conclusion, we show in a living cell nucleus that UV-modified DNA is preferentially targeted and processed by topoisomerase IIα and IIβ. This results in increased levels of topoisomerase II-mediated DNA breaks, but formation of immobile, stable topoisomerase II-DNA intermediates is not notably promoted. Inhibition of topoisomerase II activity by ICRF-187 greatly diminishes UVA-induced DNA breakage, implying topoisomerase IIα and IIβ as endogenous co-factors modulating and possibly aggravating the impact of UVA light on the genome.

Type II DNA topoisomerases cleave both strands of the DNA double helix, pass other double-stranded DNA segments through the transient gaps, and religate the DNA (1, 2). These enzymes play a rather ambivalent role in the homeostasis of the mammalian genome. They are essential for chromosome condensation and the separation of sister chromatids (3, 4). At the same time, they endanger genomic integrity by producing double-stranded DNA breaks, to the 5’-ends of which they are covalently attached. The half-life of such covalent catalytic intermediates determines whether type II topoisomerases act beneficial or detrimental (5). Certain anti-cancer agents stabilize the intermediate state, thus accumulating topoisomerase II (topo II)-linked, double-stranded DNA breaks in the genome (6). Such drugs (e.g. VM 26) are termed topo II poisons, as opposed to catalytic topo II inhibitors (e.g. ICRF-187), which prevent reopening of the topo II clamp after religation, thus stabilizing a state of the enzyme non-covalently bound to intact DNA (7). The effect of topo II poisons seems mimicked by viral DNA base modifications (8, 9) including intermediates of base excision repair (10), which also enhance DNA cleavage by topo II in vitro. Solar UVA light produces a similar spectrum of DNA base modifications via photo-oxidation processes (11) and/or direct photon-absorption (12) and, thus, could likewise modify the processing of the genome by topo II.

To test this hypothesis, we irradiated small nuclear areas of living cells expressing green fluorescent protein (GFP)-linked topo IIα or IIβ (13) with defined doses of UVA light by use of an argon ion laser of a confocal microscope. At these sites, we observed rapid, long term accumulation and reduced mobility of topo II, as well as massive DNA breakage, which could be abolished by pretreatment with the catalytic topo II inhibitor ICRF-187. Our data suggest an important role for topo II in transducing the impact of UVA light on the genome.

EXPERIMENTAL PROCEDURES

Cells and Microscopy—Fully functional chimera of topo IIα or IIβ and GFP were stably expressed at physiological levels in HEK 293 cells (13). Cultivation of transfected cells under an inverted confocal laser scanning microscope (Zeiss LSM 510) and fluorescence recovery after photobleaching (FRAP) measurement of GFP chimera were done as described in Ref. 13. For quantification, the green fluorescence signal in the irradiated area was measured and its relative fluorescence intensity \( I_{rel} \) was calculated as described previously (14). Nonlinear regression analysis of FRAP kinetics was done as described previously (15).

UVA irradiation was inflicted through a UV-permissive oil immersion objective (40×/1.3 NA Plan-NeoFluar®; Zeiss). In a cell nucleus, a circular area of 0.5-μm diameter was defined with the aid of the bleach software of the microscope and irradiated by the 364 nm line of an argon Laser. Light dose was measured at the objective tip with a LP-0101 detector head consisting of an integrated sphere with a diode (Gigahertz-Optik, Puchheim, Germany). It accumulated to \( 5 \times 10^6 \) m\(^{-2}\) when 500-fold bleached pulse at 80-miliwatt nominal laser power were applied to obtain a significant accumulation of topo II-GFP. Unless stated otherwise, experiments were carried out with this dose, which has a similar genotoxic potential as 10 kJ m\(^{-2}\) of UVB, and is about 100-fold higher than a minimal erythemal dose of natural solar UVA light (16).

Self-primed in Situ Labeling of Topo II Cut Sites (Topo-SPRINS)—

The assay was developed to visualize topo II-induced DNA strand breaks on spread chromosomes (17). In principle, it is a PRINS reaction carried out in the absence of sequence-specific primers. Instead, the required, free 3’-ends formed transiently in cleavage complexes of topo II and DNA are first irreversibly fixed and then extended by DNA polymerase in the presence of rhodamine-labeled nucleotides. To apply the assay to interphase nuclei instead of chromosomes, cells were grown on cover slides and fixed with methanol. Single-stranded DNA breaks, 1

1 The abbreviations used are: topo, DNA topoisomerase; CPD, cyclobutane pyrimidine dimer; FRAP, fluorescence recovery after photobleaching; GFP, green fluorescent protein.

[^S]: This work was supported by Grants Bo 9103-1, Bo 9103-2, and Ha 1434/13-1 from the German Research Foundation (DFG). 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.

[^S]: The on-line version of this article (available at http://www.jbc.org) contains a QuickTime movie.

This paper is available on line at http://www.jbc.org 20559
which are generated by UVA light itself (18) or the fixation procedure, were sealed with DNA ligase (Quick T4 DNA Ligase, New England Biolabs). Topo II-generated breaks are not religated, because the enzyme remains covalently attached to the 5'-end of the break. Therefore, they were the only DNA breaks to be labeled subsequently by incubating specimen for 30 min at 65 °C with 50 μl of reaction mixture (10 mM Tris-HCl, pH 8.9, 100 mM KCl, 1.5 mM MgCl2, 0.05% Tween 20, 8.7% glycerol, 100 μg/ml bovine serum albumin, 0.05% Tween 20, 8.7% glycerol, 100 μM dATP, 100 μM dCTP, 100 μM dGTP, 65 μM dTTP, 35 μM tetramethylrhodamine-5-dUTP, 2 units of Tth DNA polymerase (Roche Diagnostics)). The reaction was stopped (4× SSC, 0.05% Tween 20) and cells were then intensively washed (4× SSC, 0.05% Tween 20) and mounted for microscopy.

RESULTS AND DISCUSSION

To test the impact of UVA radiation on the disposition of topo II in living cells, we irradiated cells expressing GFP-tagged topo IIα or IIβ (13) with defined doses of UVA light. The cells were cultured under a confocal microscope, and an argon ion laser (λ = 364 nm) was focused through the objective at defined areas of cell nuclei. Monitoring of GFP fluorescence after the UVA pulse showed a long term (>1.5 h) accumulation of topo II at the UVA-exposed site (Fig. 1). Accumulation started immediately after irradiation and increased gradually until a steady state was reached after 100–150 s (Fig. 2A). The extent of the process (2–3-fold above initial nucleoplasmic level) was clearly related to light dose (Fig. 2A, inset), and its gradual onset allowed ruling out UVA-induced DNA-protein cross-linking, which occurs instantaneously. To exclude the possibility that accumulation was due to the aggregation of oxidized enzyme molecules, we repeated the experiment at mitosis, focusing the UVA laser either at the chromosomal plate (Fig. 2B, left) or the cytosol (right). If accumulation was due to aggregation of oxidized enzyme molecules, it should likewise occur in chromosomes and cytosol. However, accumulation was only seen when the focus of UVA irradiation was within the chromosomal plate not when it was directed at a cytosolic area. This difference was most notable with topo IIβ-GFP (Fig. 2B, bottom), which at mitosis is evenly distributed between chromosomes and cytosol (13). Thus, aggregation of oxidized protein clusters could be ruled out. This observation made it also unlikely that topo II was attracted to UVA lesions due to its plausible involvement in repair processes (19, 20), since these are supposed to be inactive during mitosis. Therefore, the most likely interpretation of the observed DNA dependence of topo II accumulation was an enhanced processing of UVA damaged DNA by the enzyme. We tested this assumption subsequently by analyzing the mobility of the enzyme in the UV-irradiated area using photobleaching techniques.

The mobility of some biofluorescent DNA-binding proteins has been analyzed in the living cell nucleus by nonlinear regression analysis of kinetics of FRAP. These studies revealed fast and slow states of the proteins that coexist and are interconvertible (15, 21). In the case of topo I, the slow state could be assigned to enzyme engaged in DNA turnover, since stabilization of the DNA-bound enzyme form by camptothecin increased both amount and half-time of the slow fraction, whereas the fast fraction was not affected (15). When comparing here by a similar approach the impact of DNA damage on topo IIα-GFP (Fig. 2C), we found the enzyme less mobile in UVA-exposed areas (Fig. 2C, squares) than in corresponding areas not irradiated (Fig. 2C, triangles). Upon preincubation with ICRF-187 (Fig. 2C, circles), the enzyme became even more retarded in the nucleoplasm, confirming that the catalytic inhibitor prevents dissociation from DNA subsequent to religation (7). Nonlinear regression analysis (hatched lines in Fig. 2C) indicated two fractions of fluorescent molecules with different mobility contributing to each set of data. In the normal situation (cells neither irradiated with UVA nor treated with ICRF; Fig. 2C, triangles), significantly (p < 0.002) best fits were achieved by assuming 86% of the enzyme in a fast (t1/2 = 1.3 s) and 14% in a slow state (t1/2 = 19 s). In UVA-irradiated areas (Fig. 2C, squares), the slow fraction increased to 35% and became further retarded (t1/2 = 41 s), whereas ICRF treatment (Fig. 3, circles) immobilized (t1/2 > 700 s) most of the enzyme molecules (73%). These and similar observations made with cells expressing topo IIβ-GFP (not shown) suggest that type II topoisomerases accumulate at UVA-irradiated DNA sites because there they are less mobile. The most likely interpretation of this finding is that the enzymes bind either directly to the damaged DNA or to other proteins recognizing the lesions.

Interestingly, recovery kinetics in Fig. 2C show that in the UVA-irradiated area topo II is retarded but not immobilized, since the corresponding FRAP curve (squares) fully recovered to initial intensity within about 3 min (in untreated areas recovery was complete after ~1 min). This demonstrates that UVA-induced DNA damage clearly does not promote long lasting, covalent entrapment of topo II on chromosomal DNA in the same manner, as does topo II poisoning (Fig. 2C, circles, and Ref. 13). Instead, topo II accumulation at UVA lesions obviously reflects a transient interaction, which may explain why a related assay for isolating in vitro complexes of topo II to DNA readily detects drug-induced enzyme-DNA covalent complexes (22) but not an UVB-induced association of topo II with irradiated, cellular DNA (23).

To check whether topo II was catalytically active at all in the irradiated area, we added ICRF-187 after the UVA pulse, thus trapping the enzyme preferentially in those places where it has gone through the entire catalytic cycle. Fig. 3 (and the supplementary QuickTime movie) shows that ICRF-187 greatly enhanced accumulation of topo IIα-GFP and IIβ-GFP (not shown) in the UVA-irradiated area, suggesting an increased catalytic interaction of topo II with UVA-exposed, chromosomal DNA. In the absence of ICRF-187, this enhanced activity should generate an increase in topo II-generated DNA cuts at UVA-irradiated sites, even if the catalytic cycle of the enzyme is not altered by light-induced modifications of the substrate.

To corroborate this deduction, we employed self-primed in situ extension of topo II cut sites with fluorescent nucleotides (17), which was adapted to visualize those DNA sites that are actually cleaved by topo II in an interphase nucleus. A normal result is shown in Fig. 4A, row 1; topo IIα-GFP resides mostly in the nucleoli with hardly any incorporation of rhodamine-dUTP in the nucleoplasm. Row 2 shows the effect of a typical topo II poison (VM 26). When cells were cultured with VM 26 (20 μM) for 10 min before fixation, topo IIα-GFP became almost completely de-localized from nucleoli, due to the drug trapping the enzyme in covalent DNA bonds at genomic DNA cut sites in the nucleoplasm (13). Incorporation of rhodamine-dUTP was significantly enhanced at exactly those sites, where topo II had been trapped by VM 26 (row 2, compare middle left and right...
...doses of 5, 3.5, or 2 ml of ICRF-187 (100 μM final concentration) was added to the culture medium and imaging continued for another 10 min. Top, images of an irradiated (left) and an untreated cell (right) before and after irradiation (the complete sequence of events can be viewed in the supplementary QuickTime movie). Bottom, increase in fluorescence intensity in the UVA-exposed spot plotted over time. Scale bar, 1 μm.

Our findings do not allow direct deductions as to the mechanistic impact of UVA lesions on the catalytic cycle of topo II. Previous investigations using UVC irradiation to introduce mainly cyclobutane pyrimidine dimers (CPDs) into plasmid DNA in vitro revealed that the DNA strand passage step of the enzyme on UVA-exposed, genomic DNA. A cell expressing topo II-GFP was imaged before and every 3.1 s after UVA irradiation of the indicated area. 5 min after irradiation, ICRF-187 (100 μM final concentration) was added to the culture medium and imaging continued for another 10 min. Top, images of an irradiated (left) and an untreated cell (right) before and after irradiation (the complete sequence of events can be viewed in the supplementary QuickTime movie). Bottom, increase in fluorescence intensity in the UVA-exposed spot plotted over time. Scale bar, 1 μm.

and 100 s after irradiation of a chromosomal (left) or a cytoplasmic area (right). Brackets mark metaphase chromosomes. C, FRAP analysis of cells expressing topo IIα-GFP. Cells were left untreated (top), UVA-irradiated (middle), or exposed to a final concentration of 100 μM ICRF-187 (bottom; note the nuclear de-localization of topo IIα-GFP due to drug-induced trapping of the enzyme to genomic DNA cut sites in the nucleoplasm (13)). Then, a defined area (white circles; δ = 1 μm) in the nucleoplasm of ICRF-treated or untreated cells, or in the DNA-exposed area of irradiated cells, was bleached. Top, images taken before and at selected time points after the bleach pulse; bottom, fluorescence intensities in the bleach spot plotted as relative recovery over time (Δ, untreated; □, UV-exposed spot; ○, ICRF-treated; thin solid lines, all data points; hatched lines, non-linear regression of the data).

Fig. 3. The catalytic topo II inhibitor ICRF-187 traps the enzyme on UVA-exposed, genomic DNA. A cell expressing topo IIα-GFP was imaged before and every 3.1 s after UVA irradiation of the indicated area. 5 min after irradiation, ICRF-187 (100 μM final concentration) was added to the culture medium and imaging continued for another 10 min. Top, images of an irradiated (left) and an untreated cell (right) before and after irradiation (the complete sequence of events can be viewed in the supplementary QuickTime movie). Bottom, increase in fluorescence intensity in the UVA-exposed spot plotted over time. Scale bar, 1 μm.

images), confirming that the assay visualizes topo II-linked DNA breaks. The control experiment shown in row 3, where VM 26-pretreated cells were assayed without DNA polymerase added, demonstrates that the nucleolar and cytoplasmic staining by rhodamine-dUTP is due to a nonspecific binding of the fluorescent nucleotide to these structures. In Fig. 4B (top), a confined area of the cell nucleus was irradiated with UVA before performing the reaction. As expected, the irradiated area was clearly accentuated by accumulation of green fluorescent topo IIα-GFP (middle left). However, the intense incorporation of rhodamine-dUTP (middle right) at exactly the same site of topo IIα-GFP accumulation gives a clear indication of massive DNA breakage. To ascertain that the DNA breaks were not only co-localized and coincident with topo IIα-GFP, but actually mediated by the enzyme, the cellular complement of topo II was immobilized with ICRF-187 in the non-covalently DNA-bound form prior to UVA irradiation (Fig. 4B, bottom). This prevented accumulation of topo IIα-GFP at the UVA-irradiated site (middle left image) and blocked incorporation of rhodamine-dUTP into the UVA-exposed area (middle right), indicating that rhodamine-dUTP incorporation in the absence of ICRF-187 must have been entirely due to extension of topo II-mediated DNA cuts. In other words, UVA-irradiation required active topo II as a co-factor to induce the DNA breakage detectable by the assay.

Our findings do not allow direct deductions as to the mechanistic impact of UVA lesions on the catalytic cycle of topo II. Previous investigations using UVC irradiation to introduce mainly cyclobutane pyrimidine dimers (CPDs) into plasmid DNA in vitro revealed that the DNA strand passage step of the...
Fig. 4. UVA-induced DNA breaks are mediated by top II. Cells expressing topo II-GFP were fixed, unspecific single strand breaks were labeled by DNA polymerase-mediated incorporation of rhodamine-dUTP (middle right), Corresponding phase contrast images (left), GFP fluorescence (middle left), and a merged image of GFP and rhodamine fluorescence (right) are shown. A, cells were either left untreated (top) or were treated with the top II inhibitor VM 26 for 15 min before fixation (middle and bottom). In the bottom row, DNA polymerase was omitted in addition to reveal unspecific staining.

**a**Cells were grown on a microscopic grid to enable identification of individual cells after the topo-SPRINS assay. A selected cell was exposed to UVA irradiation in a microscopic grid to enable identification of individual cells after the topo-SPRINS assay. A selected cell was exposed to UVA irradiation in the indicated area (white circle in the phase contrast image), 5 min later, cells were fixed and assayed as above. Arrowheads mark the UVA-irradiated spot. The bottom row shows a cell pretreated with 100 μM ICRF-187 for 10 min before irradiation.

catalytic cycle of Drosophila topo II is inhibited by the alteration of the DNA substrate, whereas the cleavage/preligation equilibrium is not affected (24). While CPDs are likely to contribute to UVA-induced DNA damage when introduced into selected sites in oligonucleotide substrates (9). In summary, it is clear that solar UVA irradiation leads to various DNA base modifications and that topo II cleavage when introduced into selected sites in oligonucleotide substrates (9). In summary, it is clear that solar UVA irradiation leads to various DNA base modifications and that DNA cleavage. Actually, DNA breakage at UVA-exposed DNA sites seems to a major part mediated by topo II, since it can be more or less abolished by catalytic topo II inhibitors such as ICRF-187 (Fig. 4B). Thus, topo II is indicated as an endogenous cofactor converting DNA base modifications into DNA strand breaks. This may have far reaching implications for carcinogenesis and cell aging, biological processes believed to follow from damage of cellular DNA. In reactive oxygen species generated in the course of solar UVA exposure and/or energy metabolism (26, 27). The basic chemistry of DNA modification by oxygen radicals (11) and photo absorption (12) is well established, but it is entirely unclear how such comparably minor and repairable DNA base modifications can sum up to massive degeneration of the entire genome of nuclei and mitochondria, which is the hallmark of carcinogenesis (27) and cell aging (28). Moreover, it is unclear why different tissues and cell types are affected differently by a similar burden of oxidative stress (29). If topo II was an important endogenous cofactor determining the over all effect of DNA base modifications on the genome, as implied by the data presented here, then the cellular level of topo II should determine the extent to which exposure to sunlight and oxidative stress becomes translated into genomic degeneration.

**Acknowledgments**—We are grateful to Hilde Merkert and Jörg Hacker (University Würzburg) for generously providing access to a confocal microscope, to Claus L. Andersen for expert technical advice, and to Jean Krutmann (University Düsseldorf) for helpful discussions.

**REFERENCES**

[1] Wang, J. C. (1996) Annu. Rev. Biochem. 65, 635–692
[2] Champoux, J. J. (2001) Annu. Rev. Biochem. 70, 369–413
[3] DiNardo, S., Voelkel, K., and Sternglanz, R. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 2616–2620
[4] Grue, P., Grieger, A., Schestedt, M., Jensen, P. B., Uhse, A., Straub, T., Ness, W., and Boege, F. (1999) J. Biol. Chem. 274, 33660–33666
[5] Frollich-Ammon, S., and Osheroff, N. (1995) J. Biol. Chem. 270, 21429–21432
[6] Liu, L. F. (1989) Annu. Rev. Biochem. 58, 351–375
[7] Boca, J., Ishida, R., Berger, J. M., Audib, T., and Wang, J. C. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 1781–1785
[8] Kingma, P. S., and Osheroff, N. (1998) Biochim. Biophys. Acta 1400, 223–232
[9] Sabourin, M., and Osheroff, N. (2000) Nucleic Acids Res. 28, 1947–1954
[10] Wistermann, A. M., and Osheroff, N. (2001) J. Biol. Chem. 276, 46290–46296
[11] Ravanat, J. L., Douki, T., and Cadet, J. (2001) J. Photochem. Photobiol. B Biol. 63, 88–102
[12] Rochette, P. J., Therrien, J. P., Drouin, R., Perdiz, D., Bastien, N., Drohetsy, E. A., and Sage, E. (2003) Nucleic Acids Res. 31, 2786–2794
[13] Christensen, M. O., Larsen, M. K., Barthelmes, H. U., Hock, R., Andersen, C. L., Kjeldsen, E., Knoesen, B. R., Westergaard, O., Boege, F., and Mielke, C. (2002) J. Cell Biol. 157, 31–44
[14] Ghirri, R. D., and Mustel, T. (2000) Nature 404, 609–609
[15] Christensen, M., Barthelmes, H., Feines, S., Knoesen, B., Boege, F., and Mielke, C. (2002) J. Biol. Chem. 277, 15661–15665
[16] Drohetsy, E. A., Turcotte, J., and Chateauauneuf, A. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 2352–2354
[17] Andersen, C. L., Wadall, A., Kjeldsen, E., Mielke, C., and Koch, J. (2002) Chromosome Res. 10, 305–312
[18] Douki, T., Reynaud-Angelin, A., Cadet, J., and Sage, E. (2003) Biochemistry 42, 9221–9229
[19] Hickson, I. D., Davies, S. L., Davies, S. M., and Robson, C. N. (1990) Int. J. Radiat. Biol. 58, 561–568
[20] Stenman, T., and Böhr, V. A. (1993) Carcinogenesis 14, 1841–1850
[21] Hoogstraten, D., Nigg, A. L., Heath, H., Mullenders, L. H., van Driel, R., Hoeijmakers, J. H., Vermeulen, W., and Houtsmuller, A. B. (2002) Mol. Cell 10, 1165–1174
[22] Subramanian, D., Furbee, C. S., and Muller, M. T. (2001) Methods Mol. Biol. 93, 137–147
[23] Subramanian, D., Rosenstein, B. S., and Muller, M. T. (1998) Cancer Res. 58, 976–984
[24] Corbett, A. H., Zeichdich, E. L., Lloyd, R. S., and Osheroff, N. (1991) J. Biol. Chem. 266, 16666–16671
[25] Christ, S. D., and Hanawalt, P. C. (2003) Nat. Rev. Mol. Cell. Biol. 4, 361–372
[26] Berneburg, M., Grether-Beck, S., Kurten, V., Ruzicka, T., Briviba, K., Sies, H., and Houtsmuller, A. B. (1999) J. Biol. Chem. 274, 15345–15349
[27] Erc, B. (2000) Biol. Chem. 385, 467–475
[28] Beckman, K. B., and Ames, B. N. (1998) Physiol. Rev. 78, 547–581
[29] Beckman, K. B., and Ames, B. N. (1998) Ann. N. Y. Acad. Sci. 854, 118–127
Enhanced Processing of UVA-irradiated DNA by Human Topoisomerase II in Living Cells
Christian Mielke, Morten O. Christensen, Hans Ullrich Barthelmes and Fritz Boege

J. Biol. Chem. 2004, 279:20559-20562. doi: 10.1074/jbc.C400032200 originally published online March 23, 2004

Access the most updated version of this article at doi: 10.1074/jbc.C400032200

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

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2004/04/07/C400032200.DC1

This article cites 29 references, 11 of which can be accessed free at http://www.jbc.org/content/279/20/20559.full.html#ref-list-1