Most higher organisms have a system of innate immune defense that is mediated by a group of evolutionarily related, germ line-encoded receptors, so-called Toll-like receptors. In mammals Toll-like receptors signal in response to pathogen-associated microbial structures. For example, Toll-like receptor 2 appears to mediate responses to bacterial peptidoglycan and acetylated lipoproteins and Toll-like receptor 4 to bacterial lipopolysaccharide. However, the structural principles that underlie recognition of these structures are poorly understood. Toll-like receptors have leucine-rich repeats in their extracellular domains and are thus believed to adopt solenoid structures, similar to that found in platelet glycoprotein Ib. Additionally, all Toll-like receptors contain N-linked glycosylation consensus sites, and Toll-like receptor 4 requires glycosylation for function. Toll-like receptor glycosylation is also likely to influence receptor surface representation, trafficking, and pattern recognition. Using circular dichroism spectroscopy, we show here that purified human Toll-like receptor 2 and 4 proteins have secondary structure contents similar to glycoprotein Ib. We have also analyzed where consensus glycosylation sites are located in the extracellular domains of other human Toll-like receptors. We found that there are significant differences in the location and degree of conservation between sites in different Toll-like receptors. Using site-directed mutagenesis, we have found that in Toll-like receptor 2 extracellular domain all four predicted glycosylation sites are substituted, although one site is inefficiently core-glycosylated and its removal drastically affects secretion. The remaining Toll-like receptor 2 glycosylation sites also contribute to efficient protein secretion, albeit to a lesser degree.

In humans, innate immune responses provide the first line of defense against invading bacterial pathogens. Pathogen-associated molecular patterns such as lipopolysaccharide from Gram-negative bacteria, peptidoglycan, and foreign nucleic acids are sensed by several different immune cell types such as macrophages and dendritic cells. These cells then mediate complex inflammatory and antiviral responses. Stimulation of dendritic cells by pathogen patterns is also required for T-cell maturation and the development of an adaptive immune response (see Ref. 1 for a review).

In recent years it has become clear that the human Toll-like receptors (TLRs) are required to mediate these responses. These molecules are single pass transmembrane receptors and are related to Drosophila Toll, a protein involved in dorsoventral patterning and antifungal innate immunity in the fly (2-4). Drosophila Toll and TLRs all have ectodomains with characteristic blocks of leucine-rich repeats and a cytoplasmic signaling domain of about 200 residues called the Toll/interleukin 1 receptor domain. The family of Toll receptors appears to use common components in the postreceptor signaling pathway, resulting in the activation of the transcription factor NFκB (5, 6).

An important area in TLR research is to understand the way in which pathogen patterns are able to activate these receptors at the biochemical and structural level. In the case of Drosophila Toll, pathogen patterns indirectly activate an endogenous cytokine-like ligand, Spätzle, and this dimeric protein activates signal transduction by dimerizing the Toll receptor (7). However, the human TLRs appear to have a different mechanism of activation that involves co-receptor proteins and direct sensing of the pattern. For example, signaling by TLR4 probably requires direct binding of lipopolysaccharide to the accessory co-receptor protein MD2 and consequent dimerization of the receptors (8).

To study these molecular recognition events biochemically requires the production of the receptor domains in a pure and functional form. This has proved difficult to achieve, and there are few reports of expression and purification of receptor ectodomains in the literature. The ectodomains consist primarily of leucine-rich repeat sequences (LRRs), 24-amin acid motifs that fold together into a solenoidal structure (see Fig. 1). LRRs are found in a large superfamly of proteins (5) and seem adapted for the rapid evolution of diverse protein binding specificities. Although no structures of TLRs are presently known, the crystal structures of two related LRR extracellular proteins, platelet glycoprotein Ib (9) and the Nogo receptor (10), have recently been solved in complex with specific protein binding partners. These structures show that unlike some other LRR proteins (for example, ribonuclease/angiogenin inhibitor (11)) the convex surface of the LRRs is not α-helical but of variable, extended secondary structure (see Fig. 1).

In this study we have described the expression and purification of TLR 2 and 4 ectodomains and have shown that they lack α-helical secondary structure. We have also probed the glyco-
glycosylation status of TLR2. Using site-directed mutagenesis, we found that all four predicted sites are used and that one of these, the conserved site 4, is heterogeneously glycosylated. Mutation of all the sites severely affects the biosynthesis and secretion of the TLR2 ectodomain.

EXPERIMENTAL PROCEDURES

Sequence Alignments and Bioinformatic Analysis—TLR protein sequences were retrieved from the EMBL data base using SRS (sequence retrieval system). Retrieved protein sequence accession numbers are as follows: human TLR1 Q9UC90, murine TLR1 Q9EPW5; bovine TLR2 QG6L66, chicken TLR2 variant 1 Q9DJ78, chicken TLR2 variant 2 Q9DJG6, equine TLR2 A2ARl9169_P, hamster TLR8 Q9F18S, human TLR2 O15454, macaque TLR2 Q86535, murine TLR2 Q8DBC4, pig TLR2 AAQ64238, rat TLR2 Q8BSQ7, rat TLR3 Q8N074, rabbit TLR3 P58727, chimpanzee TLR4 Q9QTN1, equine TLR4 Q9MYW3, hamster TLR4 Q9W84V, human TLR4 Q9UM57, murine TLR4 Q9Z203, rat TLR4 Q9QX05; human TLR5 O15454, murine TLR5 Q8JLF7; human TLR6 Q8Y292; monkey TLR6 Q6W7Q4, pig TLR6 QAC9817, p; human TLR7 Q9RN89, murine TLR7 Q9E311; human TLR8 Q9NYG9, murine TLR8 Q91X17; dog TLR9 Q86550, human TLR9 Q9NYC3; human TLR10 Q9GN97, pig TLR10 Q85R59; pig TLR11 AAQ19138; human TLR10 Q85RB5. For analysis of site conservation, sequences from different species were aligned using ClustalW and color-coded using Joy (12). To analyze where sites were located within the LRR solenoid structure, individual LRRs were manually aligned based on the LRR definitions of Bell et al. (13). Alignments were then colored using Joy as before. Structural representations were created using PyMol (www.pymol.org), based on the crystal structure of the Nogo receptor (10).

Cell Culture—Sf9 and Tn4 cells for baculovirus generation and protein expression were grown in suspension culture in serum-free SF900 II medium (Invitrogen) containing 1% pluronic acid (Sigma). For the expression of glycosylation mutants HEK293 (human embryonic kidney) cells were used and cultured in HEPES-modified Dulbecco’s modified Eagle’s medium (Sigma), supplemented with 10% fetal bovine serum, and antibiotics (penicillin/streptomycin).

Expression of Recombinant Toll-like Receptors 2 and 4—Expression constructs for residues 1–587 and 1–631 of the TLR2 and TLR4 extra- and transmembrane domains, respectively, were generated by PCR on a vector containing the TLR2 open reading frame using a 5′ primer encoding a BamHI site and Kozac sequence and a 3′ primer encoding a HindIII site with a multiple cloning site. Site-directed mutagenesis was carried out according to the instructions in the Stratagene QuickChange kit. The following primers (and their reverse complements) were used for mutagenesis (mutated codons in bold): N114S, atc tat aat tta ttc tct tct ttc tgc aac ggc; N114E, atc tat aat tca tta cag gac gta agt cag gtc ctc; D199E, taa ggt tgg aca att cag gac gta agt cag gtc ctc; T421G, agc gtt ctc aag aac ttc gct cta cag atc tca gta gac; A416T, tgc atc cag aaa aac ttc gct cta cag atc tca gta gac; A416G, caa aag atg tat tgg gac tta aac cga aca gta gac; D424N, caa gaa atg atg aag atg tgg AAC tta tca aag cga atc cag; D424N, caa gaa atg atg aag atg tgg AAC tta tca aag cga atc cag; D424N, caa gaa atg atg aag atg tgg AAC tta tca aag cga atc cag.

RESULTS

A Single Conserved Glycosylation Site Is Present in TLR2 and Is Located on the Conserved Face of the Leucine-rich Repeat Solenoid—Analysis of the primary sequence of TLR2 ectodomains reveals the presence of four potential glycosylation sites. All of these lie within consensuses leucine-rich repeats, and three are predicted to lie in the constant regions of the LRR (Fig. 1, A and B). Site 1 is predicted to be solvent-exposed on the convex surface of the LRR solenoid. By contrast, sites 2 and 3 are located on the concave surface but in a position predicted to be solvent-exposed and to make the asparagine residues available for modification. On the other hand, site 4 is located on the terminal residue of the parallel β-sheet of LRR16, a structure that forms part of the inner surface of the solenoid; access to this site in the folded state might be sterically restricted. We examined whether any of these four sites is conserved in the 11 TLR2 sequences currently known. As shown in Fig. 1C, only site 4 is conserved in all species; the other three are present in about 6 of 11 species.

The Ectodomains of Toll-like Receptors 2 and 4 Are Secreted in a Monomeric and N-glycosylated Form when Expressed in Insect Cell Culture—The extracellular domains of TLR2 and TLR4 were secreted from Sf9 insect culture cells infected with baculovirus expression constructs. The proteins were purified from culture supernatants using metal affinity chromatography and gel filtration chromatography (Fig. 2A). Both TLR2 and TLR4 ectodomains eluted as single peaks at positions that indicate the proteins are monomeric (Fig. 2B). To study the glycosylation status of TLR2, we treated samples with several glycosidases: PNGase F, an amidase that cleaves between the terminal GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides from complex sugars; and O-glycosidase, which releases the asparagine-linked N-glycans (sialic) acid structures from complex sugars; and O-glycosidase, which releases the disaccharide β-galactosidase (1–3)–GalNAc from O-glycans bound to serine or threonine (17). Protein samples were dena-
tured beforehand or left untreated and then incubated overnight in the presence of glycosidases at 37 °C under mildly denaturing or native conditions as indicated. Samples were analyzed by SDS-PAGE (see Fig. 2C). Both protein preparations are insensitive to treatment with neuraminidase and O-glycosidase, which suggests that there is no or very little O-glycosylation. By contrast, both endoglycosidase H and PNGase F deglycosylate TLR2, demonstrating the presence of N-linked sugars.

Circular Dichroism Spectra of TLR2 and TLR4 Indicate a Lack of α-Helical Structure—To analyze the secondary structure adopted by TLR2 and TLR4 ectodomains, we measured circular dichroism spectra of the purified proteins. As shown in Fig. 3, both samples display a negative band at -217 nm. This indicates the presence of β structure, but the lack of bands at 208 and 222 nm suggests a low content of α-helix in the proteins. The secondary structure content of the spectra was estimated using the program Selcon3 (14) and compared with known LRR protein structures (Table I). This result strongly suggests that the LRRs of TLR2 and 4 adopt an overall architecture similar to that of platelet glycoprotein Ib and the Nogo receptor (see Fig. 1) (9, 10) and unlike that of ribonuclease inhibitor, which has a high α-helical content (18).

All Four Predicted Glycosylation Sites in TLR2 Are Modified and Are Required for Efficient Secretion—To study the role of TLR2 glycosylation further, the four predicted N-linked sites were sequentially removed by site-directed mutagenesis. To conserve function and folding, sites 1 to 3 were changed to

Fig. 1. Localization and conservation of glycosylation sites in the TLR2 ectodomain. A, structural representation of the TLR2 ectodomain LRRs and flanking regions with N-linked glycosylation consensus sites. Sites located in LRR turn regions projecting laterally are colored in green; site 4, which is located in the LRR β-sheet region, is blue. B, sequence alignment of consecutive TLR2 LRRs. N-linked glycosylation sites are highlighted by black boxes. C, sequence alignments of TLR2 sequences from different species showing N-linked glycosylation sites 1–4 and surrounding sequences. Color coding of amino acids according to Ref. 28. Similar analysis for TLR4 is shown in Fig. S1 and a summary for the other TLRs in Table S1.
Fig. 2. The TLR2 and TLR4 extracellular domains can be expressed and purified from insect cell culture, and the TLR2 extracellular domain is glycosylated. A, expression and purification of TLR2 and TLR4 extracellular domains. Culture supernatants of insect cells infected with TLR2 and TLR4 recombinant baculoviruses were concentrated and purified by Ni-NTA purification (lanes 1 and 3, respectively) and gel filtration (lanes 2 and 4, respectively). Samples were analyzed on 4–20% SDS-PAGE and stained with Coomassie Blue. B, gel filtration profiles of purified TLR2 and TLR4 ectodoms. Left, purified TLR2 extracellular domain analyzed on a calibrated analytical Superdex 200 gel filtration column. Right, purified TLR4 extracellular domain analyzed on a calibrated analytical Superdex 75 gel filtration column. C, treatment of TLR2 extracellular domain protein with glycosidases. Purified TLR2 protein was treated with FNGase F (F), endoglycosidase H (H), neuraminidase (N), or O-glycosidase (O), as indicated, under native or denatured conditions overnight at 37 °C. Samples were then analyzed on 8% SDS-PAGE and Coomassie-stained.

Fig. 3. Circular dichroism spectroscopy of TLR2 and TLR4 extracellular domains. Spectra were recorded in phosphate buffer from 250 to 190 nm, duplicate runs were averaged, and a buffer control subtracted. Data were analyzed using CDPro as shown in Table I.

Table I
Selcon3 analysis of TLR2 and TLR4 CD spectra and comparison with LRR proteins of known three-dimensional structure

| Protein               | Secondary structure feature | α-Helix | β-Sheet | β-Turn |
|-----------------------|-----------------------------|---------|---------|--------|
| TLR2                  |                             | %       | %       | %      |
| TLR4                  |                             | %       | %       | %      |
| Ribonuclease inhibitor |                             | %       | %       | %      |
| Glycoprotein Ib        |                             | %       | %       | %      |
| Internalin B LRR domain|                             | %       | %       | %      |
| Ng-R                  |                             | %       | %       | %      |

residues found at those positions in TLR2 from other species (Fig. 1C), and the fully conserved site 4 was mutated to asparagine, a residue frequently found at position 6 in other extracellular LRRs. The four mutant proteins were then expressed in HEK293 cells, followed by immuno blot assay of both supernatant and cell lysate samples (Fig. 4). Each successive mutation reduced the apparent molecular mass of the recombinant protein, demonstrating that the mutations prevented the addition of N-glycans. The wild-type TLR2 protein expressed in the presence of tunicamycin, an inhibitor of N-linked glycosylation, co-migrates with mutant 4, suggesting that mutant 4 is fully unglycosylated (Fig. 4). Taken together, these results demonstrate that each of the four sites is occupied in the wild-type TLR2 ectodomain. It is noticeable that not all protein bands are single species. In the supernatant samples where bands are generally broad and fuzzy, this is hard to distinguish, but in the cell lysate blot doublet bands are discernible. Analysis of these bands using ImageJ software confirms this observation (Fig. 4B). Differences in electrophoretic mobility manifesting themselves as multiplet bands are most likely caused by inefficient core glycosylation. That there are only doublet bands and no triplet or higher order multiplets is an indication that only one of the sites is partially glycosylated. The lower doublet band in lane 11 probably corresponds to unglycosylated protein because it co-migrates with mutant 4. In other samples, upper and lower bands correspond to mono- and diglycosylated (mutant 2), di- or triglycosylated (mutant 1) and so forth. The doublet is present in wild-type and all the mutants up to mutant 3 (lanes 8–11) but disappears in mutant 4 (lane 12). This suggests that site 4, the last remaining site in mutant 3, is the partially occupied site, removal of which generates a universally unglycosylated and unsecreted product.

No Permutation of Double or Triple Glycosylation Mutants Restores Efficient Secretion of TLR2 Ectodomain—To determine whether any TLR2 ectodomain that retains a single, homogeneous glycosylation site can be efficiently secreted, the three remaining triple mutants were made. As shown in Fig. 5, none of these proteins is secreted by HEK293. However, a substantial amount of protein can be detected intracellularly, and it appears that the single glycosylation sites are used as the bands migrate more slowly than the mutant lacking all the sites. In addition, three further permutations not involving site 4 cannot be secreted (Fig. 6). Interestingly, although the single mutant lacking site 1 is secreted at only a slightly reduced level compared with wild-type, the site 4 single mutant is severely impaired. This points to the heterogeneous site 4 as being particularly critical for secretion of TLR2.

DISCUSSION

The expression studies and purification of TLR2 and TLR4 presented here show that folded and monodisperse proteins can be produced by baculovirus-infected insect cell culture cells in amounts sufficient for biochemical and structural analysis. The ability of the TLR2 ectodomain to be secreted in a heterologous expression system is consistent with the behavior of endogenous human TLR2. TLR2 is detected on the surface of mono-
cytes, and TLR2 ectodomains are shed from these cells (19). It is proposed that shed TLR2 may have a physiological role in regulating responses to pathogens.

The circular dichroism spectroscopy studies presented here indicate that the LRRs of TLR2 and TLR4 are likely to fold into an overall structure comparable with that of platelet glycoprotein Ib and the Nogo receptor. This finding has allowed us to predict the likely arrangement of the polysaccharide chains in TLR2 and TLR4 (see Fig. 1 and Supplemental Fig. S1).

We have shown that human TLR2 is glycosylated when expressed in a human cell line and thus confirmed the characteristic of TLR2 as a glycoprotein. Although glycosylation of the cell surface TLR2 receptor protein may be different (20), our data suggest that all four glycosylation sites are modified in the mature TLR2 protein. Other studies suggest that this is also the case for TLR4. TLR4 requires glycosylation for receptor function (21); its functional importance may be reflected in the high degree of conservation of TLR4 glycosylation sites, all of which are conserved (see Fig. S1). In TLR1 two of six and in TLR6 five of nine glycosylation sites are conserved across species. In TLR2, which features the least number of glycosylation sites in all TLRs, only site 4 is conserved. An analysis of

![Image](https://example.com/image1.png)

**Fig. 4.** Properties of glycosylation mutants expressed in mammalian culture cells. Immunoblot of culture supernatants (A) or cell lysates (B) from HEK293 cells transfected with TLR2 glycosylation mutants 1–4 as indicated in the absence or presence of tunicamycin (*) or transfected with empty vector (x). Samples were collected and processed 3 days posttransfection as described, separated on 8% SDS-PAGE, and probed with anti-TLR2 antibody. C, bands labeled 1, 2, and 3 were analyzed using ImageJ software.

![Image](https://example.com/image2.png)

**Fig. 5.** Monoglycosylated and glycosylated mutants of TLR2 fail to secrete. Immunoblot of culture supernatants (A) or cell lysates (B) from HEK293 cells transfected with TLR2 glycosylation mutants 4–7 as indicated or transfected with empty vector (x). Samples were collected and processed as before. The asterisk denotes a nonspecific protein also detected in the empty vector control (x).
Toll-like Receptor 2 Glycosylation

predicted glycosylation sites in all the TLRs is presented in supplementary data (Table S1). The degree of conservation observed may reflect the requirements of glycosylation in receptor function. Interestingly, the conserved site in TLR2 shows inefficient core glycosylation, whereas all other sites are efficiently substituted. Thus, this site may be of importance for protein structure or secretion rather than for signaling function. In this regard, protein binding to both platelet glycoprotein Ib and the Nogo receptor does not require glycosylation but does involve interaction with LRR residues on the concave surface of the LRR solenoid.

Comparing the TLR2 mutants generated here, the level of protein biosynthesis appears to be similar between the different mutants despite the differences in the nature and number of mutated residues. Our observations illustrate that glycosylation is an important determinant of TLR2 secretion in mammalian cells. All mutants are impaired in their level of secretion in comparison to wild-type protein, and not only entirely unglycosylated but also monoglycosylated mutants secrete poorly. The decrease in secretion between mutants 1 and 4 (Fig. 4) shows that the overall number of glycosylation sites has a strong influence on the ability of the protein to secrete, potentially by decreasing interactions of the nascent protein chain with the cellular folding machinery. Based on this data, TLR2 appears to belong to the group of glycoproteins that require processing and translocation by the cellular folding machinery. Based on this data, TLR2 protein biosynthesis appears to be similar between the different cell types.

It appears that the group of glycoproteins that require glycosylation is an important determinant of TLR2 secretion in mammalian cells. All mutants in most, if not all, glycosylation sites for secretion (22, 23).

REFERENCES
1. Janeway, C. A., and Medzhitov, R. (2002) Annu. Rev. Immunol. 20, 197–216
2. Gay, N. J., and Keith, F. J. (1991) Nature 351, 355–356
3. Belvin, M. P., and Anderson, K. V. (1996) Annu. Rev. Cell Dev. Biol. 12, 393–416
4. Imler, J. L., and Hoffmann, J. A. (2001) Trends Cell Biol. 11, 304–311
5. Buchanan, G. S., and Gay, N. J. (1996) Prog. Biophys. Mol. Bio. 65, 1–44
6. Rock, F. L., Hardiman, G., Timans, J. C., Kastlein, R. A., and Bazan, J. F. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 588–593
7. Weber, A., Tauszig-Delamasure, S., Hoffmann, J., Leibfriese, E., Gascan, H., Ray, K., Morse, M., Imler, J., and Gay, N. (2003) Nat. Immunol. 4, 794–800
8. Gangloff, M., and Gay, N. J. (2004) Trends Biochem. Sci. 29, 294–300
9. Huizinga, E. G., Tsuji, S., Romijn, R. A. F., Schipperstor, M. E., de Groot, P. G., Sixma, J. J., and Gros, P. (2002) Science 297, 1176–1179
10. He, X. L., Bazan, J. F., McDermott, G., Park, J. B., Wang, K., Tessier-Lavigne, M., He, Z. G., and Garcia, K. C. (2000) Neuron 28, 177–185
11. Kobe, B., and Deisenhofer, J. (1995) Nature 374, 183–186
12. Mizuguchi, K., Keane, C. M., Blundell, T. L., Johnson, M. S., and Overington, J. P. (1998) Bioinformatics 14, 617–623
13. Bell, J. K., Mullen, G. E., Leifer, C. A., Mazzoni, A., Davies, D. R., and Segal, D. M. (2003) Trends Immunol. 24, 528–533
14. Sreerama, N., and Woody, R. W. (2000) Anal. Biochem. 287, 252–260
15. Laskowski, R. A. (2001) Nucleic Acids Res. 29, 221–223
16. Hausmann, M., Kieselsing, S., Mestermann, S., Webb, G., Spottl, T., Andus, T., Scholmerich, J., Herfarth, H., Ray, K., Falk, W., and Rogler, G. (2002) Gastroenterology 122, 1987–2000
17. Maley, F., Trimble, R. B., Tarentino, A. L., and Plummer, T. H., Jr. (1989) Anal. Biochem. 180, 195–204
18. Kobe, B., and Deisenhofer, J. (1992) Nature 360, 751–756
19. LeBouder, E., Rey-Nores, J. E., Ruchener, N. K., Grigoriev, M., Lown, S. D., Affolter, M., Griffin, G. E., Ferrara, P., Schiffrin, E. J., Morgan, B. P., and Labata, M. O. (2003) J. Immunol. 171, 6680–6689
20. Wojczynski, B. S., Stowers-Wojczynski, M., Shukin-Krahman, S., Wunner, W. H., and Spitalnik, S. L. (1998) Glycobio 8, 121–130
21. Correia, J. D., and Ulevitch, R. J. (2002) J. Biol. Chem. 277, 1845–1854
22. Dickson, S., and Karu, P. (1978) J. Immunol. 121, 960–966
23. Taylor, A. K., and Wall, R. (1988) Mol. Cell. Biol. 8, 4197–4203
24. Assil, I. Q., and Abou-Samra, A. B. (2001) Am. J. Physiol. Endocrinol. Metab. 281, E1015–E1021
25. Dube, S., Fisher, J. W., and Powell, J. S. (1988) J. Biol. Chem. 263, 17516–17521
26. Sienkiewicz, C. F., Luo, C. C., Nakashima, M. K., Chen, S. H., Smith, L. C., and Chan, L. (1990) J. Biol. Chem. 265, 5429–5433
27. Rey, M. A., Luther, M. A., Knoppers, M. H., Neidhardt, E. A., Khondaker, S. S., Costino, M. F., Schimmel, P. A., Francis, M. A., Moebius, U., and Rothfield, B. R. (1992) J. Biol. Chem. 267, 22428–22434
28. Taylor, W. R. (1997) J. Mol. Biol. 269, 902–943
29. Uff, W., Clemetson, J. M., Harrison, T., Clemetson, K. J., and Emsley, J. (2002) J. Biol. Chem. 277, 35657–35663
30. Marino, M., Braun, L., Cossart, P., and Ghosh, P. (1999) Mol. Cell. 4, 1063–1072
Four N-linked Glycosylation Sites in Human Toll-like Receptor 2 Cooperate to Direct Efficient Biosynthesis and Secretion
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