Proc. Nat. Acad. Sci. USA
Vol. 70, No. 7, pp. 2112–2116, July 1973

Mouse Lambda-Chain Sequences

(variable regions/plasmocytomas/BALB/c mice)

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Communicated by Herman N. Eisen, April 9, 1973

ABSTRACT Amino-acid sequences of the variable regions of three lambda chains produced by plasmacytomas of BALB/c mice are compared. Two are almost certainly identical and one differs from these by three amino acids. These findings extend our earlier conclusion on the relative uniformity of sequences in this type of immunoglobulin light chain. With amino-acid sequence data on two additional lambda chains, eight mouse lambda chains studied to date are indistinguishable and four probably differ from these by one, two, or three amino acids.

We reported previously that six of 10 lambda chains produced by plasmacytomas of BALB/c mice were indistinguishable by partial sequence and composition analyses of peptides derived by enzymatic digestions of these chains. Four of the 10 lambda chains differed from these by one, two, or three amino-acid substitutions. Thus, these lambda chains exhibit a strikingly simple pattern of variability compared to human kappa and lambda chains. Not only has the contribution to variability due to polymorphism been eliminated, but BALB/c mice appear to express only one lambda variable-region subgroup, which we interpret to be coded for by a single germ-line lambda variable-region gene. The simple pattern of variability suggests that we might be analyzing antibodies in the initial stages of selection by antigen (1).

Two of the indistinguishable lambda chains, J558 and xS104, have now been compared by further sequence analysis of their variable regions, and the results strengthen the argument that they are identical. The variant lambda chains differ from the ostensibly identical lambda chains (1) only in those regions that are hypervariable (2). This is confirmed here by additional sequence data on the variable region of the variant S178. Further, two additional lambda chains have been partially sequenced and are also indistinguishable from the six apparently identical lambda chains previously reported.

MATERIALS AND METHODS

The light chains used in this study are produced by plasmacytomas of BALB/c mice. J558, W3159, S104, and S178 are tumors induced in Dr. Melvin Cohn's laboratory (Hirst, J., Jones, G., Weigert, M., and Cohn, M., unpublished). M511 was given to us by Dr. Michael Potter. TPCK-treated trypsin (EC 3.4.4.4) and α-chymotrypsin (EC 3.4.4.5) were obtained from Worthington Biochemical Corp. (Freehold, N.J.). Thermolysin, grade B, was obtained from Calbiochem (Los Angeles, Calif.), and was recrystallized according to the method described by Matsubara (3). Carboxypeptidase-A-DFP (EC 3.4.2.1) was obtained from Sigma Chemical Co. (St. Louis, Mo.).

The myeloma proteins produced by J558, W3159, and S104 tumors were IgA. These proteins were purified from the sera of tumor-bearing mice (4), and the light chains were isolated from these proteins (5). S178 produces only lambda chain, which was isolated from the urine of female BALB/c mice. M511 lambda chain was isolated from the urine of female BALB/c mice (4). xS104 is a derivative of the S104 tumor, which produces mainly lambda chain as well as small amounts of IgA. The protein used here was the urinary lambda chain.

The purified lambda chains were denatured by either performic-acid oxidation (6) or complete reduction and *S*-aminoethylation (7).

Denatured lambda chains were enzymatically digested in 0.05 M NH_3HCO_3 for 2 hr at 37° at an enzyme to substrate ratio of 1:50 w/w with the light chains at concentrations of 10–20 mg/ml. Peptides were digested in 0.05 M NH_4HCO_3 for 2 hr at 37° with the enzymes at a 0.01% w/v concentration. Enzymatic digestions were stopped by addition of an equal volume of 1 N acetic acid, and the samples were lyophilized.

Peptides resulting from enzymatic digestions were purified by electrophoresis on paper at pH 4.7, by electrophoresis followed by chromatography (8), or by gel filtration on Bio-Gel P-4 columns (0.9 × 90 cm) equilibrated with 1% formic acid. Peptides purified by electrophoresis and chromatography were detected by staining the paper with 0.02% ninhydrin in acetone and eluted with either 50% pyridine or 6 N HCl.

The amino-acid sequence of peptides was determined by the subtractive-Edman method (9) and/or the dansyl-Edman method (10). The positions of certain residues were determined by carboxypeptidase-A digestion of peptides. This enzyme was used at a concentration of 0.01% in 25 mM Tris·HCl (pH 7.5)–0.5 M NaCl (11). Aliquots were withdrawn at different intervals of digestion and analyzed for free amino acids.

Complete acid hydrolysis was done in 6 N HCl for 16 hr at 105° in the presence of 4% thioglycolic acid (12). Partial acid hydrolysis was done either in 6 N HCl for 30 min at 105° or in 0.03 N HCl at 105° for 12 hr.

Amino-acid analyses were performed on Beckman/Spinco analyzers, models 120C and 121.

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TABLE 1. *Tryptic peptides from the variable region of J558, xS104, and S178 lambda chains*

Peptide	Residues	Protein	Amino-acid sequence
T-1	1-23	J558, xS104, S178	PCA (Ala_2, Val_3, Thr_6, Glx_3, Ser_2, Pro, Gly, Leu_2, Cys) Arg*
T-2	24-56	J558,xS104	Ser-Ser-Thr (Gly_4, Ala_2, Val_2, Thr_4, Ser, Asx_5, Tyr, Trp, Glx_2, Lys, Pro, His, Leu_2, Phe, Ile) Arg*
		S178	Ser-Asn-Thr (Gly_3, Ala_2, Val_2, Thr_4, Ser, Asx_6, Tyr, Trp, Glx_2, Lys, Pro, His, Leu_2, Phe, Ile) Arg*
T-3	57-63	J558, xS104, S178	Ala-Pro-Gly-Val-Pro-Ala-Arg*
T-4	64-72	J558, xS104, S178	Phe-Ser-Gly-Ser-Leu-Ile-Gly-Asx-Lys*
T-5	73-90	J558, xS104, S178	(Ala_4, Leu, Thr_3, Ile_2, Gly, Glx_3, Asx, Tyr, Phe) Cys*
T-6	91-105	J558,xS104	Ala-Leu-Trp-Tyr-Ser-Asx-His (Trp, Val, Phe, Gly_3, Thr) Lys*
T-6a	91-97	S178	Ala-Leu-Trp-Tyr-Ser-Asx-Arg*
T-6b	98-105	S178	Trp-Val-Phe-Gly-Gly-Gly-Thr-Lys*
T-7	106-113	J558, xS104, S178	Leu-Thr-Val-Leu-Gly-Glx-Pro-Lys*

(→) Indicates subtractive-Edman and/or dansyl-Edman.

* Indicates that the position of these residues is inferred from the known action of trypsin on lysine, arginine, and aminoethylcysteine residues.

RESULTS

The partial sequences of three lambda chains, M511, W3159, and S104, were established by the methods described (1). By these methods these light chains are indistinguishable from the xS104 and J558 light chains.

Fig. 1 shows the tentative sequence of the variable region of the xS104, J558, and S178 lambda chains. The order of the variable-region tryptic peptides (Table 1) was established by certain chymotrypsin or thermolysin peptides isolated by electrophoresis and chromatography of digests of *S*-aminoethylated lambda chains. The relevant peptides are the chymotrypsin peptides C-4, C-9, C-10, and C-20 (Table 2) and the thermolysin peptide Th-19 (Table 3). This order of tryptic peptides as inferred from the composition of these peptides is supported by the homology of these sequences and the revised sequence of the mouse lambda chain MOPC-104E (Appella, personal communication). By a comparison with the complete sequence of MOPC-104E (13), all but the region between residues 175 and 183 was accounted for by the amino-acid compositions of peptides detected after electrophoresis and chromatography of either trypsin or thermolysin digests of *S*-aminoethylated S178. The electrophoretic and chromatographic position of the peptides arising from residue 114 to the carboxy-terminus of xS104, J558, and S178 were indistinguishable. Differences between these proteins in this region that do not alter the electrophoretic and chromatographic position of a peptide or that occur between residue 175 and 183 (not detected by these procedures) cannot be excluded.

The following methods were used for sequencing the tryptic peptides that could be ascribed to the variable region by comparison with the lambda chains of MOPC-104E (13).

T-1 (residues 1–23)

Two peptides could be detected after electrophoresis and chromatography of the tryptic digest of the *S*-aminoethylated S178 protein only after they were stained by chlorination (14). One of these peptides could also be detected by staining the paper for arginine-containing peptides (15). By analogy with the results obtained with *S*-aminoethylated MOPC-104E (13), it was assumed that these peptides represented residues 1–22 and 1–23 of the 178 protein. For sequence analysis, this peptide was obtained by electrophoresis at pH 4.7 of tryptic digests of performic acid-oxidized protein. Guide strips were stained with ninhydrin and for arginine-containing peptides. T-1 was detected as a ninhydrin-negative, arginine-positive peptide. The peptide was eluted from the unstained portion of the paper, lyophilized, and digested with either thermolysin or chymotrypsin. These digests were subjected to electrophoresis at pH 4.7, and guide strips were stained with ninhydrin and by chlorination. Peptides that could be detected by ninhydrin staining were eluted, analyzed for total amino-acid composition, and sequenced by the subtractive-Edman procedure and by carboxypeptidase-A digestion. Thermolysin peptides (Table 3) were ordered by the peptides obtained after chymotrypsin digestion of T-1 (Table 2). Peptide Th-1 resulting from thermolysin digestion and C-1 from chymotrypsin digestion of T-1 could be detected only by staining of guide strips by chlorination. The amino-acid composition of Th-1 was glutamic acid and alanine. It was assumed that this peptide represented the first two amino acids of T-1 and that the first residue of the lambda chains was pyrrolidone carboxylic acid.

T-2 (residues 24–56)

This peptide could be obtained in small amounts after electrophoresis and chromatography of digests of *S*-aminoethylated protein. In order to obtain larger quantities of this peptide, digests were fractionated by gel filtration. The appropriate fraction was further purified by electrophoresis at pH 4.7.

The amino-acid sequence of the first three residues of this peptide was determined by the dansyl-Edman method.

Thermolysin or chymotrypsin digests of T-2 were subjected to electrophoresis at pH 4.7 followed by chromatography. The sequences of certain of the peptides resulting from these digestions were determined. The chymotrypsin peptide C-5 (Table 2) was found to have an NH_2-terminal sequence

J558, xS104, and MOPC-104E
1 5 10 15 20 25
Pca-Ala-Val-Val-Thr-Glx-Glx-Ser-Ala-Leu-Thr-Thr-Ser-Pro-Gly-Glx-Thr-Val-Thr-Leu-Thr-Cys-Arg-Ser-Ser-

S178
Asn-

J558, xS104, and MOPC-104E
30 35 40 45 50
Thr-Gly-Ala-Val-Thr-Thr-Ser-Asx-Tyr-Ala-Asx-Trp-Val-Glx-Glx-Lys-Pro-Asp-His-Leu-Phe-Thr-Gly-Leu-Ile-

S178

J558, xS104, and MOPC-104E
55 60 65 70 75
Gly-Gly-Thr-Asx-Asx-Arg-Ala-Pro-Gly-Val-Pro-Ala-Arg-Phe-Ser-Gly-Ser-Leu-Ile-Gly-Asx-Lys-Ala-Ala-Leu-

S178
Asn

J558, xS104, and MOPC-104E
80 85 90 95 100
Thr-Ile-Thr-Gly-Ala-Glx-Thr-Glx-Asx-Glx-Ala-Ile-Tyr-Phe-Cys-Ala-Leu-Trp-Tyr-Ser-Asx-His-Trp-Val-Phe-

S178
Arg

J558, xS104, and MOPC-104E
105 110
Gly-Gly-Gly-Thr-Lys-Leu-Thr-Val-Leu-Gly-Glx-Pro-Lys

S178

FIG. 1. *The tentative sequence of the variable region of mouse lambda chains.* Equivalent sequences were found for lambda chains xS104 and J558. This was also the case for most of the S178 lambda chain as indicated by a *line.* Positions at which S178 differed in sequence from xS104 and J558 are indicated by the residue involved. The revised sequence of the MOPC-104E urinary lambda chain is indistinguishable from xS104 and J558 (Appella, E., personal communication). At several positions (designated Asx or Glx) the sequencing procedures used did not permit the distinction between asparagine and aspartic acid or glutamine and glutamic acid. The sequences of the light chains are assumed to be identical at these positions, however, since the electrophoretic mobilities of the corresponding peptides from each protein, including MOPC-104E (1), that contain these residues are identical.

identical to the NH_2-terminal sequence of the first three residues of the T-2 peptide (Table 1) and could thus be placed at position 24–34. The order of the thermolysin and the chymotrypsin peptides was established by their respective overlaps (Tables 2 and 3). By the subtractive-Edman procedure, it was only possible to establish the sequence of the first three residues of the chymotrypsin peptide C-7 (residues 38–46) or the thermolysin Th-9 (residues 38–45). The rest of the sequence was established by analysis of certain peptides resulting from partial acid hydrolysis of peptide Th-9 (Table 4). The lysine at position 41 is resistant to cleavage by trypsin.

S178 differs from ×S104 and J558 by asparagine at position 25 instead of serine, and asparagine at position 52 instead of glycine. It is most likely that the amino-acid substitution in both cases is to asparagine rather than to aspartic acid, since the electrophoretic mobilities of chymotrypsin peptides C-5 and C-8 (×S104 and J558) are identical to those of the corresponding chymotrypsin peptides C-5 and C-8 from the S178 light chain. It cannot be excluded that the substitution at 52 in S178 is to aspartic acid since a second substitution in peptide C-8 from aspartic acid to asparagine at either position 54 or 55 would neutralize the expected

TABLE 2. *Chymotrypsin peptides used to establish the sequence and order of the tryptic peptides from the variable region of J558, xS104, and S178 lambda chains*

Tryptic peptide	Peptide	Residues	Protein	Amino-acid sequence
T-1	C-1	1-10	J558, xS104, S178	PCA (Ala_2, Val_2. Thr, Glx_2, Ser, Leu).
	C-2	11-20	J558, xS104, S178	Thr-Thr-Ser-Pro-Gly (Glx_2, Thr_2, Val, Leu)
	C-3	21-23	J558, xS104, S178	Thr (Cys, Arg)
T-1 and T-2	C-4	21-34	J558,xS104	(Thr_4, Cys, Arg, Ser_3, Gly, Ala, Val, Asx, Tyr)
			S178	(Thr_4, Cys, Arg, Ser_2, Gly, Ala, Val, Asx_2, Tyr)
	C-5	24-34	J558,xS104	Ser-Ser-Thr-Gly-Ala-Val-Thr-Thr-Ser-Asx-Tyr
			S178	Ser-Asx-Thr-Gly-Ala-Val-Thr-Thr-Ser-Asx-Tyr
T-2	C-6	35-37	J558, xS104, S178	Ala-Asx-Trp
	C-7	38-46	J558, xS104, S178	Val-Glx-Glx (Lys, Pro, Asx, His, Leu, Phe)
	C-8	47-56	J558,xS104	Thr-Gly-Leu-Ile-Gly-Gly-Thr (Asx_2, Arg)
			S178	Thr-Gly-Leu-Ile-Gly-Asx-Thr (Asx_2, Arg)
T-2, T-3 and T-4	C-9	55-64	J558, xS104, S178	(Asx, Arg_2, Ala_2, Pro_2, Gly, Val, Phe)
T-4 and T-5	C-10	65-75	J558, xS104, S178	(Ser_2, Gly_2, Leu_2, Ile, Asx, Lys, Ala_2)
	C-12	73-75	J558, xS104, S178	(Ala_2, Leu)
	C-13	76-88	J558, xS104, S178	Thr-Ile-Thr-Gly (Ala_2, Glx_2, Thr, Asx, Ile, Tyr
	C-14	73-90	J558, xS104, S178	(Thr_3, Ile_2, Gly, Ala_2, Glx_3, Asx, Tyr, Phe, Cys
	C-15	91-93	J558, xS104, S178	(Ala, Leu, Trp)
	C-16	94-98	J558,xS104	Tyr-Ser-Asx (His, Trp)
	C-17	94-97	S178	Tyr-Ser-Asx-Arg
	C-18	99-100	J558, xS104, S178	(Val, Phe)
	C-19	101-105	J558, xS104, S178	(Gly_3, Thr, Lys)
T-6 and T-7	C-20	101-106	J558, xS104, S178	(Gly_3, Thr, Lys, Leu)

(→) Indicates subtractive-Edman.

TABLE 3. *Thermolysin peptides used to establish the sequence and order of the tryptic peptides from the variable region of J558, xS104, and S178 lambda chains*

Tryptic peptide	Peptide	Residues	Protein	Amino-acid sequence
T-1	Th-1	1-2	J558, xS104, S178	(Glx, Ala)
	Th-2	3-9	J558, xS104, S178	Val-Val-Thr-Glx-Glx-Ser-Ala
	Th-3	10-17	J558, xS104, S178	Leu-Thr-Thr-Ser-Pro-Gly-Glx-Thr
	Th-4	18-19	J558, xS104, S178	Val-Thr
	Th-5	20-23	J558, xS104, S178	Leu-Thr (Cys, Arg)
T-2	Th-6	24-28	J558, xS104	Ser-Ser-Thr-Gly-Ala
			S178	Ser-Asn-Thr-Gly-Ala
	Th-7	29-31	J558, xS104, S178	Val (Thr_2)
	Th-8	32-37	J558, xS104, S178	(Ser, Asx_2, Tyr, Ala, Trp)
	Th-9	38-45	J558, xS104, S178	Val-Glx-Glx (Lys, Pro, Asx, His, Leu)
	Th-10	46-48	J558, xS104, S178	Phe-Thr-Gly
	Th-11	49-56	J558, xS104	Leu-Ile-Gly-Gly-Thr (Asx_2, Arg)
			S178	Leu-Ile-Gly-Asn-Thr (Asx_2, Arg)
T-5	Th-12	73-74	J558, xS104, S178	Ala-Ala*
	Th-13	75-76	J558, xS104, S178	(Leu, Thr)
	Th-14	77-84	J558, xS104, S178	Ile-Thr-Gly-Ala-Glx-Thr-Glx-Asx
	Th-15	85-86	J558, xS104, S178	Glx-Ala
	Th-16	77-86	J558, xS104, S178	(Ile, Thr_2, Gly, Ala_2, Glx_3, Asx)
	Th-17	87-88	J558, xS104, S178	Ile-Tyr
	Th-18	89-90	J558, xS104, S178	Phe-Cys
T-5 and T-6	Th-19	89-91	J558, xS104, S178	Phe (Cys, Ala)
T-6	Th-20	92-94	J558, xS104, S178	(Leu, Trp, Tyr)
	Th-21	95-98	J558, xS104	Ser-Asx (His, Trp)
	Th-22	95-97	S178	Ser-Asx-Arg
	Th-23	99-105	J558, xS104, S178	Val-Phe-Gly-Gly-Gly (Thr, Lys)

(→) Indicates subtractive-Edman, (←) carboxypeptidase-A.

* Ala-Ala dipeptide has a different electrophoretic and chromatographic mobility than free alanine.

electrophoretic difference. However, since the chymotryptic peptide, C-9 (55–64), is identical in all three cases, such a second substitution is unlikely.

T-3 (residues 57–63), T-4 (residues 64–72), and T-7 (residues 106–113)

These peptides were obtained from electrophoresis and chromatography of trypsin digests of *S*-aminoethylated protein. The peptides were eluted from paper, and the sequence was determined by the subtractive-Edman procedure.

T-5 (residues 73–90)

T-5, from tryptic digests of *S*-aminoethylated lambda chain, was purified by gel filtration followed by electrophoresis at pH 4.7. The order of the peptides resulting from thermolysin digestion was established by chymotrypsin peptides.

T-6 (residues 91–105)

This peptide was isolated by gel filtration of tryptic digests of the *S*-aminoethylated J558 and xS104 proteins, and sequenced by the subtractive-Edman procedure. Thermolysin or chymotrypsin peptides of T-6 were separated by electrophoresis at pH 4.7 and sequenced by the same procedure. The order of the chymotrypsin peptides was established by the peptides obtained by thermolysin digestion.

T-6a (residues 90–97) and T-6b (residues 98–105)

T-6 was absent in the trypsin digest of *S*-aminoethylated S178, being replaced by T-6a and T-6b. T-6a and T-6b were isolated by gel filtration, sequenced by the subtractive-Edman procedure, and ordered by their homology with T-6 from J558 and xS104.

TABLE 4. *Partial acid hydrolysis and amino-acid sequence of thermolysin peptide Th-9 (residues 38–45) of J558, xS104, and S178 lambda chains*

Peptide*	Residues	Amino-acid composition and sequence	Amino-acid sequence†
A-1	43	Free aspartic acid	
A-2	38-42	(Val_1, Glx_2, Lys_1, Pro_1)	
A-3	44-45	His-Leu	Val-Glx-Glx-Lys-Pro-Asp-His-Leu
B-1	43	Free aspartic acid	
B-2	39,40	Free glutamic acid	
B-3	40-41	(Glx_1, Lys_1)	
B-4	44-45	(His_1, Leu_1)	

(→) Indicates subtractive-Edman.

* A-1, A-2, and A-3 are peptides derived from partial acid hydrolysis in 0.03 N HCl at 105° for 12 hr. B-1, B-2, B-3, and B-4 are peptides derived from partial acid hydrolysis in 6 N HCl at 105° for 30 min.

† Combined data from Tables 3 and 4.

DISCUSSION

With the addition of lambda chains W3159 and M511 to the previous study (1), eight of 12 mouse lambda chains compared are indistinguishable in amino-acid sequence of peptides that, on the basis of comparison with MOPC-104E lambda chain, correspond to the entire variable segment (positions 1–113). This conclusion, based previously on partial sequences and amino-acid compositions of peptides, is strengthened by the more extensive sequence data presented here on the variable regions of three of the repeat lambda chains. Two of these, xS104 and J558, (shown here) are identical to the revised sequence of MOPC-104E lambda chain (Appella, E., personal communication). The four variant mouse lambda chains are indistinguishable from the eight repeat lambda chains in all but the hypervariable regions that seem to determine the combining site. This has been confirmed here for one variant chain by the complete sequence of the variable region of S178.

We have interpreted these observations to mean that the identical sequences are coded for by a single germ-line variable-region gene and the variant lambda chains, such as S178, by somatic variants of this gene. The finding of lambda chains such as RPC-20 and S176 that have one amino-acid replacement (accountable for by single base chainges) implies that antigenic selection can act on the products of single-step mutants of a germ-line gene. The S178 lambda chain that differs probably only by three amino-acid replacements (accountable for by four base changes) from the repeat sequence supports the idea that antigenic selection operates sequentially on amino-acid replacements in specificity-determining regions (1).

If myeloma tumors arose from antibody-producing cells previously selected for by antigen, the expression of identical sequences before diversification can be explained if the product of a germ-line variable-region gene in combination with an appropriate heavy chain had been selected. This possibility was suggested from the finding that two of the myeloma proteins associated with lambda chains, J558 (Hirst, J., Jones, G., Weigert, M., and Cohn, M., unpublished) and MOPC-104E (16) have specificity for the α-1,3 glucosyl linkage in dextran. As shown in Fig. 1, the λ chain, isolated from the IgAλ, J558, is identical to the urinary λ chain produced by tumor MOPC-104E, which is indistinguishable from the λ chain associated with the IgM myeloma protein also produced by this tumor (Appella, E., personal communication). That the lambda chain indeed contributes to the α-1,3 glucosyl linkage specificity of these proteins is likely since the anti α-1,3 antibody elicited by immunization of BALB/c mice with dextran is exclusively associated with lambda chains (17), even though this light-chain class represents only 3–5% of the normal light-chain population in mice (18).

As other myeloma proteins associated with this lambda chain, such as S104 and W3159 described here or J698 and H2061 (1), do not have α-1,3 glucosyl linkage specificity, this specificity is not solely due to the germ-line lambda chain, and must result from a specific heavy- and light-chain interaction. Thus, the association of the germ-line lambda chain with different heavy chains is likely to result in antibodies with different specificities. Selection for these specificities as well as for anti-α-1,3 glucosyl linkage specificity could explain the high frequency of myelomas with germ-line lambda chains.

This work was supported by research grants from the National Institutes of Health (A-105875) and a Training Grant (CA-05213) to Dr. Melvin Cohn, and no. 18263 to M.W. I.M.C. was supported by Fogarty International Fellowship no. 3 F05 TW01608-01S1, and M.W by an American Cancer Society Faculty Research Award No. PRA-59. We thank Dr. Melvin Cohn for his helpful discussions and advice and Mrs. Cheryl Look and Miss Shirlee Yonkovich for their excellent technical assistance.

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