Chromosomal Translocations Joining LCK and TCRB Loci in Human T Cell Leukemia

By Benjamin Tycko*, Stephen D. Smith†, and Jeffrey Sklar*

From the *Division of Diagnostic Molecular Biology, Department of Pathology, Brigham and Women’s Hospital, and Harvard Medical School, Boston, Massachusetts 02115; and the †Department of Pediatrics, University of Chicago, Chicago, Illinois 60637

Summary
A case of T lymphoblastic leukemia (T-ALL) showing t(1;7)(p34;q34) as the sole karyotypic abnormality was investigated at the molecular level. Screening of a phage library of tumor DNA with a probe for the beta T cell receptor gene (TCRB), which maps to chromosomal band 7q34, resulted in the isolation of a clone containing DNA spanning the translocation breakpoint of the der(1) chromosome. This clone contained chromosome 1 DNA juxtaposed upstream of a D/3-JB joint. Cloning of the corresponding germline region of chromosome 1 resulted in the isolation of a phage containing the breakpoint from the reciprocal, der(7), product, which showed chromosome 1 DNA joined downstream to a Vβ segment. Comparison of germline and translocation clones demonstrated that breakage of chromosome 1 had occurred at the border of a tandem repeat of Alu sequences. To search for transcripts from DNA near the breakpoint, a chromosomal walk was initiated along chromosome 1. A probe consisting of chromosome 1 DNA from 24-30 kb upstream of the breakpoint hybridized to a transcript derived from the gene encoding the lymphocyte-specific tyrosine kinase p56lck, previously mapped to chromosomal band lp34. The nonrandom nature of the breakpoints in this case was confirmed by the analysis of a second independent case of T-ALL containing a t(1;7) translocation, which was also found to show breakage within the LCK locus. The chromosomal breakpoint in the first case was localized 2 kb upstream of the lck upstream promoter and first nontranslated exon, while the breakpoint of the second case lay between the two alternative lck promoters, upstream of the second exon. Relative to normal thymus and activated T cells, levels of lck mRNA were greatly elevated in the first case and moderately elevated in the second. The existence of these translocations raises the possibility that alterations in the promoter region of the LCK locus may play a role in human cancer.
leukemia (T-ALL) and a cell line, SUPT12, derived from this case. We have found that the translocation in SUPT12, and a similar t(1;7) translocation in a second T-ALL cell line, HSB-2, both result in the juxtaposition of the LCK locus on chromosome 1 to the TCRB locus on chromosome 7.

Materials and Methods

Cell Lines. The SUPT12 cell line was derived from a case of T-lineage ALL (13). The cells were grown under hypoxic conditions as previously described (14). The karyotype, showing t(1;7)(p34;q34) as the only abnormality, and the pattern of TCRB gene rearrangements of this cell line are both concordant with that present in the original tumor (reference 14 and B. Tycko and J. Sklar, unpublished observations). The HSB-2 cell line, which also shows a T-ALL phenotype (15), contains a t(1;7)(p34;q34) as the only karyotypic abnormality (16). Cells were obtained from the American Type Culture Collection (Bethesda, MD).

Genomic Clones and Probes. Restriction maps of phage clones and the positions of probes are shown in Fig. 1. DNA surrounding the translocation breakpoint of the der(1) chromosome in SUPT12 cells was cloned from a library of SUPT12 DNA in phage λ590 screened with a mixed (JB1 + JB2) TCRB J-region probe (17). Positive clones were tested for chromosome 1 sequences by using restriction fragments as probes on Southern blots of DNA from a human/hamster hybrid cell line (kindly provided by E. Stanbridge, University of California, Irvine, CA) containing human chromosome 1 but lacking human chromosome 7. This showed that one set of clones consisted of chromosome 1 DNA juxtaposed upstream of JB sequence (clone A). To isolate the germline chromosome 1 DNA which had been interrupted by the translocation, a chromosome 1 probe (probe A, a 1.2 kb Sacl-HindIII fragment) derived from clone A was used to screen a genomic library constructed in λ590 with HindIII-digested germ line (sperm) DNA. This resulted in the isolation of clone A2. The probe also derived from this phage (probe B, a 1.0 kb Sacl-HindIII fragment) was used to screen another library of BamHI-digested SUPT12 DNA (constructed in vector λ47). This resulted in the isolation of clone A3, containing the breakpoint of the der(7) chromosome. To obtain clones corresponding to additional chromosome 1 DNA, probe A was used to screen a library of EcoRI-digested germ line DNA (constructed in vector λ47), resulting in the isolation of clone A4. Probe C, a 0.5 kb AluI-EcoRI fragment derived from clone A4, was in turn used to screen a library of normal human fibroblasts (partial Sau3A digest; constructed in phage λFIX; Stratagene Corp., La Jolla, CA), resulting in the isolation of clone A5. Probe D was isolated as an approximately 5.5 kb BstEII-EcoRI fragment of clone A5. The kkb coding region probe was a 2 kb cDNA containing the entire coding region of the gene (18). The GAPDH probe was a 1.4 kb cDNA (19). The Cβ probe was a 2 kb EcoRI-HindIII fragment containing the human Cβ1 region (20).

DNA Sequencing. Restriction fragments spanning the breakpoint regions of clones A1, A2 and A3 were subcloned in M13 mp18 and mp19 sequencing vectors. Nucleotide sequences were determined by dideoxy chain-termination using T7 DNA polymerase (Sequenase; United States Biochemical Corp., Cleveland, OH).

Southern Blotting. DNA was purified by proteinase K/SDS digestion and phenol extraction. DNA samples, 6 μg, were digested with restriction enzymes and analyzed by electrophoresis on 0.8% agarose gels followed by blot transfer to nylon membranes, prehybridization and hybridization with 32P-labeled DNA probes at high stringency as previously described (21).

Northern Blotting and Densitometry. RNA was isolated by cellysis in guanidinium isothiocyanate and cesium chloride gradient centrifugation. RNA samples, approximately 1.5 μg of oligo-dT-selected (poly-A+) RNA, were analyzed by electrophoresis on 1% agarose gels. Blots were hybridized with 32P-labeled DNA probes at high stringency as previously described (21). Densitometry was performed on a Microscan 1000 gel analyzer (Technology Resources, Inc., Nashville, TN).

Results

Chromosomal Translocation t(1;7)(p34;q34) in SUPT12 Cells Joins Chromosome 1 DNA to the TCRB Locus. Since the TCRB locus lies at band 7q34, we suspected that the translocation in SUPT12 might have resulted from joining of chromosome 1 DNA to a Dβ or Jβ segment on chromosome 7. Accordingly, a phage library of tumor DNA was screened with a TCRB J-region probe. Positive clones were analyzed for the presence of chromosome 1 sequences by using unique-sequence fragments as probes on Southern blots of DNA from human/mouse hybrid cells containing human chromosome 1 but lacking human chromosome 7. One set of clones, presumably corresponding to the translocation breakpoint region, consisted of chromosome 1 DNA juxtaposed upstream of Jβ sequence.

Restriction mapping of a presumptive translocation breakpoint clone (Fig. 1; A1) was used to localize the site of divergence from TCRB sequence. A restriction fragment spanning this region was sequenced (Fig. 2 a). The 3' end of this sequence consisted of a Dβ1.1-Jβ2.7 joint while the 5' end consisted of sequence not recognizable derived from Vβ DNA or TCRB germline DNA upstream of Dβ1.1, and therefore likely to be derived from chromosome 1. To localize the exact site of breakage relative to chromosome 1, germline chromosome 1 DNA was cloned by using a probe generated from the 5' (chromosome 1) end of the original phage clone (Fig. 1; probe A) to screen a phage library of germline DNA. Mapping of the resulting germline clone (Fig. 1; A2) showed that it spanned 5.5 kb of chromosome 1 DNA centered on the site of breakage. A probe from the 3' end of this clone (Fig. 1; probe B), which hybridized to a rearranged band in Southern blots of tumor DNA, was used to screen a second tumor DNA library, leading to the isolation of a third clone (Fig. 1; A3) encompassing the breakpoint of the der(7) chromosome; that is, the reciprocal product of the translocation.

Restriction fragments spanning the breakpoint region in each of the three clones were sequenced. The der(7) product consisted of a TCRB V-region (Vβ3h16 of reference 22) joined upstream of chromosome 1 sequence (Fig. 2 A). Alignment of the der(7), germline chromosome 1, and der(1) sequences (Fig. 2 A) showed that the translocation was essentially balanced with respect to chromosome 1; only 3 base pairs of DNA had been lost (Fig. 2 A). This was not true for chromosome 7, where the DNA between Vβ3h16 and Dβ1.1 had been deleted. Based on these features the translocation could most simply be conceptualized, from the standpoint of chromosome 7, as having occurred during a failed attempt at V-D.

1 Abbreviation used in this paper: T-ALL, T lymphoblastic leukemia.
rarrangement in which endonucleolytic attack by the lymphocyte recombinaise had taken place normally at Vβ and Dβ heptamer-nonamer signals (23) but in which the resulting free coding ends had failed to unite. Consistent with this were short G/C-rich stretches of apparently randomly added nucleotides at the sites of breakage and rejoining and a short exonucleolytic deletion into the 5' V-region coding sequence — both features characteristically found at normal coding joints in TCR gene rearrangements (23).

Previously it has been suggested that, at least in some classes of lymphoid tumor-associated translocations, the site of breakage within the non-antigen receptor gene as well as within the antigen receptor locus might be explained as resulting from recombinaise activity (24–26). This is supported by the presence in some translocations of ectopic heptamer-like sequences close to the sites of breakage in or around the non-antigen receptor locus (24–26, 27). We therefore searched for sequences similar to the canonical heptamer CACTGTG in the germline chromosome 1 sequence around the breakpoint. None were found. However, examination of chromosome 1 sequence (Fig. 2 A and B) showed that breakage had occurred 34 bp downstream from the 3' end of Dβ1.1. However, there has been a loss of 3 bp from the 3' end of D81.1. Furthermore, there has been a loss of 3 bp from the 3' end of D81.1 and 4 bp from the 3' end of β2.7 in formation of the downstream D-J joint. (B) Long-range structure of the translocation breakpoint region in SUPT12. As shown in (A), the translocation breakpoint is situated approximately 34 bp downstream of the 3' end of the second directly repeated Alu motif. The borders of the three Alu half-repeats which were sequenced are indicated by bars. The DNA upstream and downstream of this cluster of Alu elements in the germline chromosome 1 is unique in sequence, as established by the lack of hybridization of the corresponding restriction fragments to a total human DNA probe and by the fact that these restriction fragments hybridized to unique bands on Southern blots of genomic DNA. The position of the lck first exon is indicated.

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![Restriction maps of human chromosomes 1 and 7 surrounding the chromosomal breakpoints of t(1;7)(p34;q34) translocations.](image)

Figure 1. Restriction maps of human chromosomes 1 and 7 surrounding the chromosomal breakpoints of t(1;7)(p34;q34) translocations. The TCRβ locus on chromosome 7 (27) is oriented in the usual 5' to 3' (left to right) transcriptional direction. This places the LCK locus on chromosome 1 in an inverted orientation, with the direction of transcription from right to left. Chromosome 1 DNA is shown as a plain line; chromosome 7 DNA is shown as a bold line. For both chromosomes the position of the centromere is at the left. Phage clones are designated λ through λ5: λ1 contains the der(1) breakpoint region of the SUPT12 translocation cloned in λ590 vector, λ2 contains the corresponding region of the germline (GL) chromosome 1 clone in λ590, λ3 contains the der(7) breakpoint region of the SUPT12 translocation cloned in λ47, λ4 and λ5 contain germline chromosome 1 DNA upstream of the breakpoint cloned in λ47 and λFIX, respectively. Probes discussed in the text are indicated by bold lines. The lck first and second exons are indicated by shaded boxes. The positions of the SUPT12 and HSB-2 translocation breakpoints relative to chromosome 1 DNA are indicated by the arrows. Restriction enzyme sites are designated as H = HindIII, S = Sall, R = EcoRI, and B = BamHI. "Position of this site is ambiguous." Phage isolated from a Sau3AI

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Figure 2. (A) Sequence of DNA spanning the der(1) and der(7) breakpoints and the region of chromosome 1 interrupted by the translocation in SUPT12. Appropriate restriction fragments spanning 500 to 400 bp upstream and downstream of the breakpoint in der(1), germline chromosome 1, and der(7) clones were sequenced. Only that part of the sequence immediately surrounding the breakpoints is shown. Chromosome 7 sequence is shown in bold letters; chromosome 1 sequence is shown in plain letters. Regions of sequence identity are indicated by dots. Stretches of random nucleotides inserted at the sites of breakage are designated N and shown in italics. The three nucleotides (AAC) lost from chromosome 1 in the translocation are bracketed. The sequence of chromosome 1 is shown starting with an uninterrupted stretch of 19 A residues, representing the 3' end of the last in a set of two directly repeated Alu elements. Although the germline sequence of Vβh16 is not available, there appears to have been a loss of at least 3 bp from the 3' end of the this segment, as deduced from conserved sequences in the series of TCRB cDNA clones in reference 22, in the process of translocation. No residues have been lost from the 5' end of Dβ1.1. However, there has been a loss of 3 bp from the 3' end of D81.1 and 4 bp from the 3' end of β2.7 in formation of the downstream D-J joint. (B) Long-range structure of the translocation breakpoint region in SUPT12. As shown in (A), the translocation breakpoint is situated approximately 34 bp downstream of the 3' end of the second directly repeated Alu motif. The borders of the three Alu half-repeats which were sequenced are indicated by bars. The DNA upstream and downstream of this cluster of Alu elements in the germline chromosome 1 is unique in sequence, as established by the lack of hybridization of the corresponding restriction fragments to a total human DNA probe and by the fact that these restriction fragments hybridized to unique bands on Southern blots of genomic DNA. The position of the lck first exon is indicated.
Figure 3. Southern analysis of DNA from cell lines bearing the t(1;7)(p34;q34). Total genomic DNA, 5 μg, from SUP-T12 and HSB-2 cell lines and placenta (GL), was digested with the indicated restriction enzymes. Hybridization was carried out with the indicated chromosome 1 and chromosome 7 (Cβ) probes. With chromosome 1 probe A, rearranged bands are seen in SUP-T12 in BgIII, EcoRI and HindIII digests (a rearrangement in the BamHI digest is not resolved from the germline band in this blot). With this probe HSB-2 DNA shows rearrangements in the EcoRI and BamHI digests. Rearrangements are also present in an EcoRI digest of HSB-2 DNA hybridized with an kK first exon probe, chromosome 1 probe C and the Cβ probe. The upper rearrangement seen in HSB-2 cells with chromosome 1 probe C is in the identical position as the upper rearrangement seen with the Cβ probe, confirming the juxtaposition of LCK and TCRB loci in this case.

which had been reported to show a t(1;7)(p34;q34) translocation as the sole structural abnormality by karyotypic analysis (16), displayed rearranged bands in Southern blot autoradiograms with several different restriction enzymes and using two different chromosome 1 probes (Fig. 3). To more completely characterize this second case and to search for transcripts derived from this region of chromosome 1, we isolated two additional overlapping phage clones (Fig. 1; X4 and X5) spanning about 30 kb of DNA upstream of the breakpoint of the original case. Southern analysis using probes derived from these clones showed that the site of breakage of chromosome 1 in HSB-2 cells lay within a HindIII fragment between 4 and 11 kb upstream of the breakpoint in SUP-T12. With respect to chromosome 7, the cytogenetic assignment of the breakpoint to 7q34 suggested that the translocation in HSB-2 had also interrupted the TCRB locus. To confirm this, a Southern blot of HSB-2 DNA digested with EcoRI was hybridized sequentially with a chromosome 1 probe (Fig. 1; probe A) and a probe for the TCRB constant region. One of the rearranged bands seen with the TCRB probe coincided with the single rearranged band seen with the chromosome 1 probe (Fig. 3).

The SUP-T12 and HSB-2 Translocations Join the TCRB Locus to the LCK Locus. To identify a possible transcript from DNA near the chromosome 1p34 breakpoint, RNA from a panel of cell lines and tissues of both lymphoid and non-lymphoid origin was subjected to Northern analysis with probes derived from each chromosome 1 phage clone. The only probe which detected a major transcript was the 5' EcoRI-EcoRI fragment of the X5 clone (Fig. 1; probe D). The transcript was about 2.2 kb in size, was seen in T-ALL cell lines regardless of the presence or absence of the t(1;7)(p34;q34), and was absent from nonlymphoid sources. In view of the fact that the kK gene maps to band 1p34, shows lymphoid-specific expression, and gives rise to a 2.2 kb mRNA, these features suggested that the transcript derived from the LCK locus. Consistent with this interpretation, rehybridization of the Northern blots to a bona fide kK cDNA probe gave a superimposable pattern of bands (not shown).

Transcripts of the LCK locus originate from two alternative promoters, positioned 5' of the first (nontranslated) and second (translated) kK exons, respectively (10, 28). To confirm the localization of the translocation breakpoints to the vicinity of the kK gene and to determine the positions of breakage...
of the two cases relative to the \( \text{llk} \) exons, the restriction maps of the chromosome 1 clones were compared to the published maps of DNA in the vicinity of the first and second \( \text{llk} \) exons (28). The maps were identical in these regions, fixing the positions of the \( \text{llk} \) exons relative to the overlapping set of \( 1p34 \) clones. Based on this map, the first case had broken upstream of the first exon and the second case had broken between the first and second exons (Fig. 2). This was further confirmed by sequencing a 1 kb DNA restriction fragment (Fig. 1; probe A) lying 2 kb upstream of the breakpoint cloned from the first case, containing a region predicted to correspond to the \( \text{llk} \) upstream promoter and first exon. The sequence of the 5' half of this fragment matched the published sequence of a 600 bp EcoRI-HindIII fragment immediately upstream of the \( \text{llk} \) first exon (28). In addition to localizing the two translocation breakpoints with respect to the first and second \( \text{llk} \) exons, the map of the chromosome 1 clones physically links the two exons and shows, by alignment with the previously published local restriction maps, that the two exons are separated in the germline by about 24 kb of DNA. Our estimation of this distance is somewhat smaller than that recently reported by others (29).

Knowledge of the structure of the SUP-T12 translocation allows orientation of LCK on chromosome 1. Given the known transcriptional orientation of the TCRB gene on chromosome 7 as centromere - 5' TCRB 3' - telomere (30), the 5' to 5' (head-to-head) juxtaposition of TCRB and \( \text{llk} \) genes in the t(1;7)(p34;q34) establishes the transcriptional orientation of the LCK locus within the p-arm of chromosome 1 as telomere - 5' LCK 3' - centromere.

**LCK mRNA Expression in HSB-2 and SUP-T12 Cells.** Both examples of the t(1;7)(p34;q34) bring the \( \text{llk} \) promoter region into the vicinity of the TCRB constant region, with its associated transcriptional enhancer (31). By analogy with effects observed in other translocations involving antigen receptor genes (32), this factor might be expected to lead to alterations in the level of \( \text{llk} \) transcription. To address this possibility, we compared the level of \( \text{llk} \) mRNA in the two T-ALL lines carrying the translocation with the levels in three other control T-ALL lines showing comparable phenotypes but lacking the translocation (Fig. 4). As a normal control, 24-h PHA-stimulated T cell RNA was examined (in other blots normal thymus RNA was used as a control and gave comparable results). A band corresponding to \( \text{llk} \) message of normal size was observed in all five of the T-ALL cell lines, regardless of the presence or absence of the translocation. MOLT4, CEM and SUP-T3 cells all expressed high levels of \( \text{llk} \) message, from two- to seven-fold elevated over normal T cells. HSB-2 cells expressed seven-fold elevated levels. SUP-T12 cells expressed even higher amounts of \( \text{llk} \) message, 80-fold elevated over normal T cells.

**Discussion**

The \( \text{llk} \) gene encodes a protein tyrosine kinase, p56\(^{llk}\), which is normally restricted to lymphoid cells and which is considered to play a crucial role in transduction of mitogenic signals from T cell antigen receptors (18). We have shown that a recurrent translocation t(1;7)(p34;q34) in human T-lineage ALL results in the juxtaposition of the LCK locus on chromosome 1 with the TCRB locus on chromosome 7. In each of the two cases which we have examined, the site of DNA breakage with respect to chromosome 1 lies within the 5' noncoding region of the \( \text{llk} \) gene. In SUP-T12 cells the chromosome 1 breakpoint lies about 2 kb upstream of the first \( \text{llk} \) exon while in HSB-2 cells the site of breakage lies somewhat downstream, between the first and second exons. In both cases, the translocation interrupts the TCRB locus and brings about the physical linkage of the LCK locus with Cβ sequences and their associated transcriptional enhancer elements (40).

To investigate the mechanism underlying the SUP-T12 translocation, we have cloned and sequenced DNA surrounding the breakpoints of der(1) and der(7) chromosomes and the corresponding germline DNA of chromosome 1. The positioning of the der(1) breakpoint immediately upstream of a Dβ-Jβ sequence in chromosome 7 DNA and the der(7) breakpoint immediately downstream of a Vβ sequence suggests that the original translocation event may have occurred during a failed attempt at V-D-J joining, in the tumor progenitor cell. Although the lymphocyte recombinase probably participates in establishing the site of DNA breakage within chro-
mosome 7, the fact that no heptamer/nonamer-like recombinase recognition sequences were found near the site of breakage on chromosome 1 suggests that additional factors may have played a role in generating the translocation. In particular, the positioning of the breakpoint in SUP-T12 within 34 bp of the poly-dA/dT stretch at the 3' end of a tandem directly repeated Alu sequence is reminiscent of several previous examples of chromosomal rearrangements with breakage at the border of Alu or other repetitive sequences (selected examples in references 33–38). This feature may have mechanistic implications related to a presumed transposon-like function of these repetitive elements (39). However, given the high frequency of Alu elements in the genome, their association with some chromosomal breakpoints could also be a simple coincidence.

Expression of p56\(^{kk}\) appears to be regulated in normal thymocytes and T cells at least in part at the transcriptional level (28, 29, 40). The existence of recurrent chromosomal translocations involving the LCK and TCRB loci suggests the possibility that altered or deregulated expression of \(kk\) mRNA might contribute to cellular transformation. Certain virally-induced murine thymic lymphomas which have been shown to carry proviral insertions in 5' \(kk\) noncoding sequences overexpress both \(kk\) message and p56\(^{kk}\) protein (3, 4, 10, 41). In both cell lines bearing the t(1;7) translocation analysis of \(kk\) mRNA levels by Northern blotting indicates that the gene is expressed as an abundant transcript of normal size. Relative to normal thymocytes and a T-ALL cell line lacking the t(1;7) translocation, SUP-T12 cells appear to markedly overexpress the gene, while HSB-2 cells, which show a different chromosome 1 breakpoint, show only moderately elevated levels of \(kk\) mRNA. The HSB-2 cell line was included in a previous study of \(kk\) expression in a series of T-ALL cell lines which employed a combination of Northern blotting, ribonuclease protection, and primer extension experiments (42). Our findings are in agreement with the results of those studies, which also showed that total \(kk\) message in HSB-2 cells, while high compared to normal T cells, is only slightly elevated compared to other T-ALL lines. In contrast to HSB-2, the translocation in SUP-T12 has occurred upstream of the distal \(kk\) promoter. The much more dramatic (80-fold) elevation of \(kk\) message levels in SUP-T12 cells suggests the possibility of activation of this promoter, which is not normally highly utilized (28).

Our data suggest that chromosomal translocation may represent one mechanism for increased \(kk\) activity in transformed T cells. However, since many cases of T-ALL which lack relevant translocations, or at least cell lines derived from these neoplasms, show elevated \(kk\) transcription, chromosomal translocation is probably only one of several mechanisms capable of producing higher levels of \(kk\) mRNA. Future studies are indicated to assess whether cases of T-ALL which lack gross rearrangements of the LCK locus show more subtle genetic or epigenetic differences, or around the \(kk\) promoter region. Additionally, since regulation of p56\(^{kk}\) kinase activity appears to be complex, with both transcriptional, translational (41) and posttranslational controls, including protein phosphorylations, it will be necessary to measure the amount and activity of p56\(^{kk}\) in T-ALL cells directly at the protein level before forming conclusions about the effect of transcriptional changes.

We thank G. Inghirami and J. Krolewski for control RNA samples and H. Coyle and P. McFall for expert technical support.

This work was supported by NIH grants CA-38621 to J. Sklar, CA-42106 to S. Smith, and GM-43572 to B. Tycko.

Address correspondence to Benjamin Tycko, Department of Pathology, Columbia University College of Physicians and Surgeons, New York, NY 10032.

Received for publication 27 March 1991 and in revised form 19 June 1991.

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