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Variable and constant regions are separated in the 10-kbase transcription unit coding for immunoglobulin κ light chains

(ultraviolet transcription mapping/recombinant DNA probes/primary transcript/nuclear RNA splicing)

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ABSTRACT **UV transcription mapping with recombinant DNA probes containing immunoglobulin κ light chain mRNA sequences has been used to determine the size of the transcription unit coding for κ light chain mRNA and to establish the arrangement of variable and constant regions in this transcription unit. In relation to ribosomal RNA standards, the transcription of κ light chain constant region sequences into nuclear RNA exhibits a UV target size of 9.6 kbases (kb). The κ light chain variable region exhibits a UV target size of 7.6 kb indicating that it is separated by approximately 2.0 kb from the constant region in the κ light chain transcription unit. The size of the primary transcript (i.e., the direct, unprocessed RNA product of transcription) predicted from the constant region target size concurs with our previous pulse-labeling results which showed that the largest presumptive nuclear RNA precursor to κ light chain mRNA is approximately 10 kb. In addition, the UV target size of cytoplasmic κ mRNA is indistinguishable from the target size of constant region sequences in nuclear RNA. These results suggest that the κ light chain transcription unit is copied directly into a 10-kb nuclear RNA precursor in which the κ variable and constant regions are separated by approximately 2 kb. Accordingly, it is proposed that the joining of immunoglobulin κ light chain variable and constant regions occurs in the post-transcriptional processing of this large nuclear RNA precursor into κ light chain mRNA.**

Considerable evidence now suggests that immunoglobulin polypeptide chains are specified by two genes; variable region genes coding for the amino-terminal portion, and constant region genes coding for the carboxy-terminal portion (reviewed in refs. 1 and 2). It is now established from nucleotide sequence analysis that variable and constant regions are contiguous in immunoglobulin mRNA directing the synthesis of antibody polypeptide chains (3, 4). Until recently, it was postulated that variable and constant region genes are joined through rearrangements in the DNA of lymphoid cells committed to the production of specific immunoglobulin chains (reviewed in refs. 5 and 6). Brack and Tonegawa (7) have now isolated a DNA fragment containing variable and constant regions from a λ light-chain-producing myeloma cell line. In this cloned DNA, presumably containing the λ light chain structural gene sequences that are expressed in these cells, the λ variable and constant regions are separated by 1.25 kbases (kb). Recombinant clones of cellular DNA containing both variable and constant regions of immunoglobulin κ light chains or heavy chains have not yet been reported. However, hybridization studies by Rabbitts and Forster (8) suggest that variable and constant regions may also be separated in the DNA of myeloma cells producing κ light chains. Intervening sequences separating the structural gene or coding sequences in cellular DNA are not unique for immunoglobulins. Such regions were first discovered in adenovirus (9–13). Intervening sequences have now been found in various eukaryotic cellular genes including globin (14, 15), ovalbumin (16, 17), a fraction of *Drosophilia* ribosomal RNA genes (18–20), and yeast tRNAs (21–23).

UV transcription mapping has emerged as a powerful approach for mapping transcription units (i.e., DNA regions transcribed from a single promoter or initiation site) in eukaryotic cells (reviewed in ref. 24). This procedure involves the random introduction of transcription terminating UV lesions in DNA. These lesions result in the release of growing nascent RNA chains at the UV damaged site without affecting RNA chain initiations (24). Within a transcription unit, UV irradiation causes an exponential decrease in transcription with increasing distance from the initiation site. UV transcription mapping has been used to define the size of the transcription units for cellular heterogeneous nuclear RNA (hnRNA) and mRNA (25, 26) and rRNA (27, 28) as well as for early (26, 29) and late (30, 31) adenovirus mRNA. We have used UV transcription mapping to define the size of the transcription unit coding for κ light chain messenger sequences and to locate the variable and constant region sequences within this transcription unit. We find that the κ constant region target size corresponds to the size of the largest presumptive nuclear RNA precursor to κ light chain mRNA previously detected in pulse labeling experiments (32). Furthermore, we find that κ light chain variable and constant regions are not contiguous in myeloma cell DNA, but rather are separated by approximately 2.0 kb. This approach provides a relatively rapid means of mapping the arrangement of mRNA coding sequences within the transcription units of specific eukaryotic genes.

MATERIALS AND METHODS

Growth and labeling of P3 cells (MOPC 21; IgG_1, κ) have been described (33), as has the preparation of hnRNA and cytoplasmic RNA (32, 33). UV irradiation of cells prior to labeling was carried out as described by Hackett and Sauerbier (27). Briefly, 10 ml of P3 cells at a density of 3×10^6 cells/ml in phosphate-buffered saline were placed in a 10-cm petri dish and irradiated in the dark for various times (at a UV lamp incident dose of 7.5 erg/sec·mm^2 at 18 cm) with constant swirling at room temperature. The cells were diluted 1:10 into Dulbecco's modified Eagle's medium (Flow) containing 10% horse serum and shielded from visible light until they were harvested. Twenty minutes after irradiation, the cultures were labeled for 15 or 60 min with 150–200 μCi of [^{3}H]uridine per ml. Control P3 cells were carried through the same procedures except for irradiation. The extracted labeled RNA was resuspended in 1 mM EDTA/10 mM 2-{[Tris(hydroxymethyl)methyl]amino} ethanesulfonic acid (Tes), pH 7.6/0.1% sodium dodecyl sulfate and partially hydrolyzed with 0.2 M NaOH for 20 min at 0°C.

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Abbreviations: kb, kbases or kbase pairs; hnRNA, heterogeneous nuclear RNA.

It was then neutralized with Hepes. Hybridization to DNA bound to nitrocellulose filters was carried out as described (32).

The National Institutes of Health Recombinant DNA Molecule Program Advisory Committee has approved κ light chain mRNA clones pL21-1 and pL21-5 for P2 EK1 containment. The variable region-specific probe pL21-1V was generated by recloning the designated *Hpa* II fragment from κ light chain mRNA plasmid pL21-1 (Fig. 1). This fragment was inserted at *Pst* I site in the Amp^r locus of plasmid vector pBR 322 (34, 35).

RESULTS

Light Chain Variable and Constant Region Probes. Recombinant DNA clones containing MOPC 21 κ light chain mRNA sequences were constructed in pMB9 by using procedures established for the cloning of rabbit globin mRNA (35). Fig. 1 shows the restriction maps of two κ light chain mRNA recombinant clones (pL21-1 and pL21-5) generated in this original cloning. The κ light chain mRNA sequences in these two clones have been identified by the presence of multiple restriction sites predicted from the published nucleotide sequences for the MOPC 21 κ mRNA constant region (4, 36) and by the correspondence of nucleotide sequences determined in these clones with the published amino acid sequence (37) for the MOPC 21 light chain (38). Recombinant clone pL21-1V contained the first 160 nucleotides of the variable region (Fig. 1). The nucleotide sequence determined from the internal *Alu* I/*Hae* III fragment from pL21-1V exactly corresponds to the MOPC 21 κ light chain variable region amino acid sequence through Lys-24. Light chain mRNA recombinant clone pL21-5 was used as the constant region probe. This recombinant plasmid contains approximately 600 nucleotides of κ light chain mRNA sequences including the entire constant region and less than 100 nucleotides of the κ light chain variable region.

UV Mapping of Light Chain Transcription Unit. We initially determined the effects of UV irradiation on the products of the well-characterized transcription unit coding for the 45S RNA precursor of 18S and 28S RNA to provide standards in which the distance from the promoter or initiation site is established. The sensitivities of ribosomal 45S and 32S precursors and cytoplasmic 18S and 28S rRNAs are plotted in Fig. 2. The transcription unit coding for the 45S ribosomal RNA precursor in mouse is 13.9 kb (39). The 32S and 28S rRNAs mapped at 8.9 kb from the initiation site, whereas the 18S rRNA mapped at 4.4 kb. These values are in excellent agreement with the reported rRNA transcription units for mouse cells determined by UV transcription mapping and other gene mapping techniques (27, 39, 40).

Next, we determined the effect of UV irradiation on the transcription of κ light chain variable and constant region sequences into rapidly labeled nuclear RNA. Samples of P3 cells were exposed to different doses of UV irradiation, incubated 20 min to permit previously initiated nascent RNA chains to be completed, and then labeled for 15 min with [^{3}H]uridine. Provided that the variable and constant regions are contiguous in the κ light chain transcription unit as they are in the mRNA (3, 4), the UV sensitivities of these two κ mRNA regions would be experimentally indistinguishable. Instead, we found clearly different UV sensitivities for the transcription of these κ mRNA regions into hnRNA (Fig. 2). Labeled hnRNA hybridization with the constant region probe (pL21-5) exhibited a UV target size, relative to 45S pre-rRNA (13.9 kb), of 9.6 kb. Nuclear RNA hybridization with the variable region probe (pL21-1V) exhibited a UV target size of 7.6 kb in relation to the 45S pre-rRNA standard.

The UV target size of the constant region sequences in hnRNA is in excellent agreement with the size of the 40S (approximately 10 kb) nuclear RNA precursor detected in pulse-labeling studies (32). Both the κ variable and constant regions are present in the same 40S precursor molecules as evidenced by the coselection of variable region sequences when large hnRNA was isolated by hybridization with constant region DNA (Table 1). This suggests that κ variable and constant region sequences are both contained in the same 10-kb transcription unit. Accordingly, it appears that κ variable and constant regions are separated by approximately 2 kb in the 10-kb κ light chain transcription unit.

UV Mapping of Transcription Unit for Cytoplasmic κ Light Chain mRNA. If the κ light chain mRNA in the cytoplasm were derived from this large transcription unit, it should

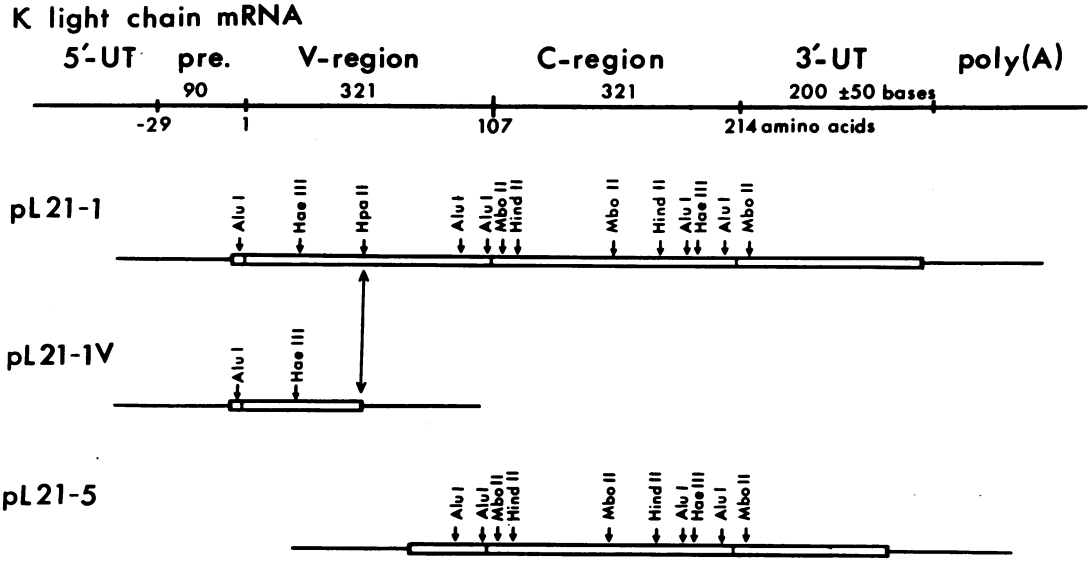

FIG. 1. Recombinant DNA probes containing κ light chain mRNA sequences. Restriction maps of κ light chain plasmids pL21-1, pL21-5, and pL21-1V are presented in relation to their location in κ light chain mRNA. Recombinant plasmids pL21-1 and pL21-5 were cloned in pMB9 (35). The variable region-specific plasmid pL21-1V was derived from a *Hpa* II fragment of pL21-1 recloned in PBR322. Only the inserted κ light chain mRNA sequences are shown in detail (□). Not all restriction sites mapped in the κ light chain mRNA sequences are shown. Flanking d(A-T) insertion sequences and plasmid vector sequences are shown by thin lines (—).

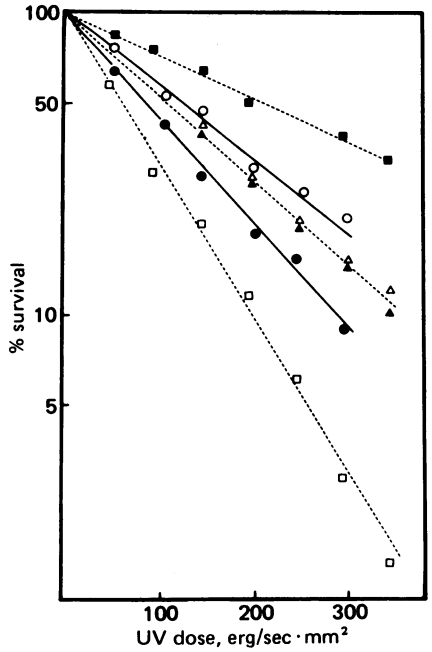

FIG. 2. UV mapping of the transcription units for κ light chain and rRNA. The survivals of radioactively labeled 32S (▲) and 45S (□) rRNA precursors and 28S (△) and 18S (■) cytoplasmic rRNA species are plotted versus UV dose. The effect of UV irradiation on the transcription of κ light chain was determined by hybridization of 15-min-labeled nuclear RNA to nitrocellulose filters containing 20 μg of variable region (pL21-1V) DNA (○) and to nitrocellulose filters containing 20 μg of constant region (pL21-5) DNA (●). Hybridization was scored after T_1 and pancreatic RNase digestion. The recoveries of hnRNA from each UV dose were normalized to the amount of 32S pre-rRNA in each sample. Each pL21-5 point is the average of six determinations. The standard deviation for each point was not greater than 3% with the exception of the 200 erg/sec·mm² point which has an SEM of 7%. Each pL21-1V point is the average of two determinations. The SEM for each point is not greater than 5%. The lines were determined by linear regression. The r of pL21-5 = 0.96, the r of pL21-1V = 0.95. The accuracy of the target size determined from these hybridizations is ±0.2 kb.

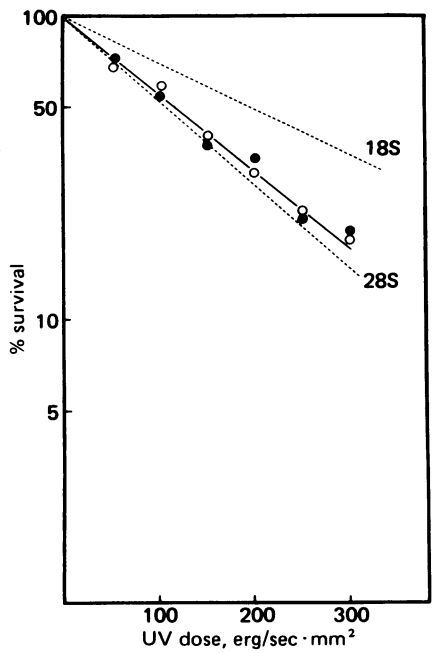

FIG. 3. The effect of UV irradiation on 60-min-labeled nuclear and cytoplasmic RNA hybridized with constant region pL21-5 DNA. Total nuclear (○) and cytoplasmic (●) RNAs were extracted from control and UV irradiated P3 cells. Each sample was then hybridized to nitrocellulose filters containing 20 μg of pL21-5 DNA. The amount of hybridization was scored after T_1 and pancreatic RNase digestion. Recoveries for each sample were normalized for nuclear RNA to 32S pre-rRNA and for the cytoplasmic RNA to 18S and 28S rRNAs.

exhibit the same UV sensitivity as the most distal sequence in the nuclear RNA required for the production of the mRNA. The UV sensitivities of the pL21-5 constant region sequences in both nuclear and cytoplasmic RNAs labeled for 60 min were indistinguishable (Fig. 3). In comparison to rRNA standards, constant region sequences in both 60-min hnRNA and cytoplasmic κ mRNA showed a UV target size of 8.4 kb. This was less than that seen in hnRNA labeled for 15 min (Fig. 2). The results in Fig. 4 compare the UV target sizes of constant region sequences in hnRNA labeled for 15 min with those in hnRNA incubated for 60 min and then labeled for 15 min. The UV target size of hnRNA decreased with increased equilibration time after UV irradiation. We attribute this apparent reduction in the target size, estimated in relation to rRNA in longer labeling periods, to a dark repair system identified in mouse cells. This system, studied by Ali and Sauerbier (41), reportedly acts on UV-damaged DNA within 1 hr after irradiation and is caffeine insensitive. The net effect of such repair is the apparent reduction in UV target size in comparison to rRNA standards which are reportedly unaffected by the repair system because several investigators show constant labeling of rRNA in mouse cells up to several hours after UV irradiation (25–27). UV studies that measure the sensitivities of cytoplasmic mRNAs or protein species may be limited by this phenomenon. To accurately measure the size of transcription units in mouse cells, one apparently needs to determine the sensitivities of hnRNA sequences during short labeling times when this effect appears to be minimal.

Table 1. Light chain 40S nuclear RNA precursor contains both constant and variable regions

Input	Hybridized cpm†		pL21-1V/pL21-5	
cpm*	pL21-5	pL21-1V	Observed	Predicted‡
28,500	1260	375	0.29	0.26

* Labeled 32–45S hnRNA from P3 cells (9×10^6 ^{3}H cpm) was hybrid-selected on 100 μg of pL21-5 DNA on nitrocellulose filters in 50% formamide/0.6 M NaCl/0.01 M Tes, pH 7.6/0.001 M EDTA/0.1% sodium dodecyl sulfate at 37°C for 12 hr. Hybridized hnRNA was not RNase digested before elution. Elution was carried out in 98% formamide at 65°C. The eluted RNA was treated with RNase-free DNase and hybridized to nitrocellulose filters containing 20 μg of either pL21-5 or pL21-1V DNA (32).

† Hybridized cpm were scored after T_1 and pancreatic RNase digestion (32). The RNase-resistant hybridized counts were enriched from 0.02% of the total input to 5.7% in the eluate, representing a 285-fold enrichment from hybrid selection.

‡ The predicted ratio of hybridized pL21-1V cpm to pL21-5 cpm was generated from the ratio of κ light chain mRNA sequences in pL21-1V to those in pL21-5 = 160/600 = 0.26.

DISCUSSION

These UV mapping studies reveal several major features of the immunoglobulin κ light chain genes undergoing transcription in myeloma cells. It appears that the size of the transcription unit in DNA coding for κ light chain mRNA is approximately 10 kb. This is a minimum estimate because sequences beyond 10 kb in the transcription unit, which are not represented in the recombinant DNA hybridization probes employed, would not be detected. However, because the UV target size for κ constant

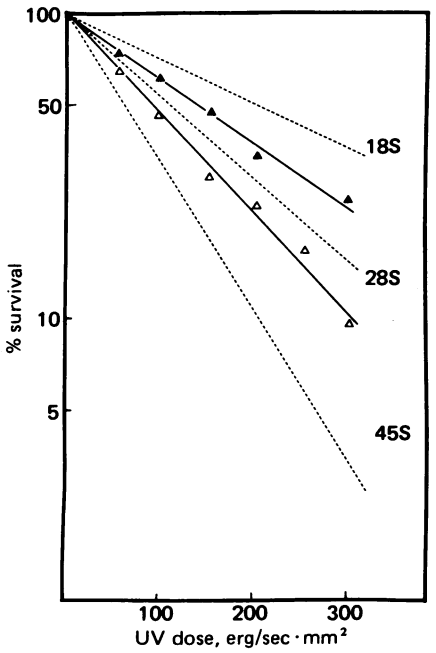

FIG. 4. The reduction in UV target size with increasing time after irradiation. P3 cells were exposed to different doses of UV irradiation, incubated either for the standard 20-min equilibration time (△) or for 60 min (▲), and then labeled for 15 min. Isolated nuclear RNA was hybridized to filters containing 20 μg of constant region (pL21-5) DNA. Hybridization was scored after T_1 and pancreatic RNase digestion. The transcription of constant region sequences in labeled nuclear RNA from cells incubated for 20 min shows a UV target size of 9.6 kb. The same sequences labeled in nuclear RNA after a 60-min incubation show a UV target size of 7 kb with a SEM of 7% for the line.

region transcription into hnRNA is in excellent agreement with the size of the largest presumptive nuclear RNA precursor detected in previous labeling experiments (32), we propose that this represents a reliable estimate of the entire transcription unit. This proposal is further strengthened by the correspondence of the UV target size of cytoplasmic light chain mRNA with that of constant region sequences in hnRNA, which suggests that κ mRNA is derived from a 10-kb transcription unit. This correspondence in UV target size also suggests that the constant region and adjacent untranslated sequences lie at, or very near, the 3′ terminus of the κ light chain transcription unit. The different UV target sizes for the synthesis of variable and constant region sequences in hnRNA indicate that these regions are separated by approximately 2 kb in the light chain transcription unit. Finally, the separated variable and constant regions appear to be present in a 5′ → 3′ orientation like that in the mRNA.

The model in Fig. 5 presents the MOPC 21 κ light chain transcription unit. In addition to a single κ constant region per haploid genome, it is now evident that multiple κ variable regions must be present in germ line DNA (reviewed in refs. 1 and 42). It is not known whether other κ variable regions are present in this 10-kb transcription unit. However, the UV mapping results reported here suggest that the transcription unit does not contain other κ variable regions related to the MOPC 21 κ variable region expressed in these P3 cells. Otherwise such related variable region sequences would have generated complex or multiphasic UV inactivation curves. In addition, the hybridization results of Rabbitts (8, 42) indicate that few, if any, κ variable regions in cellular DNA give appreciable cross hybridization with the MOPC 21 κ variable region.

The 40S nuclear RNA species is shown as the primary transcript in which variable and constant regions are separated to the same extent as in the DNA transcription unit. Of the various mechanisms proposed for joining structural gene sequences separated by intervening sequences (reviewed in ref. 43), the most plausible involves splicing of separated RNA sequences transcribed directly into a primary hnRNA transcript. Nuclear RNA splicing in the generation of cytoplasmic mRNA from widely separated sites in DNA is now established for adenovirus (9–13, 44–47) and SV40 (48, 49) and for β-globin mRNA for which it has been recently shown that the isolated 15S β-globin nuclear RNA precursor contains two intervening sequences separating β-globin mRNA coding sequences (50). Accordingly, it is predicted that κ light chain variable and constant regions that are contiguous in κ light chain mRNA (3, 4) are joined by an intramolecular splicing event in the post-transcriptional processing of the 10-kb nuclear RNA precursor. Preliminary results suggest that at least one additional splicing event, involving sequences near the 5′ terminus of κ mRNA that map near the promoter of the κ light chain transcription unit, is involved in the biogenesis of κ light chain mRNA.

Although the nature of the splicing/processing intermediates is not specified in the model (Fig. 5), pulse-labeling experiments (32) and other UV transcription studies (unpublished results) establish a 24S nuclear RNA processing intermediate (approximately 3–4 kb long) in the pathway to 13S κ light chain mRNA.

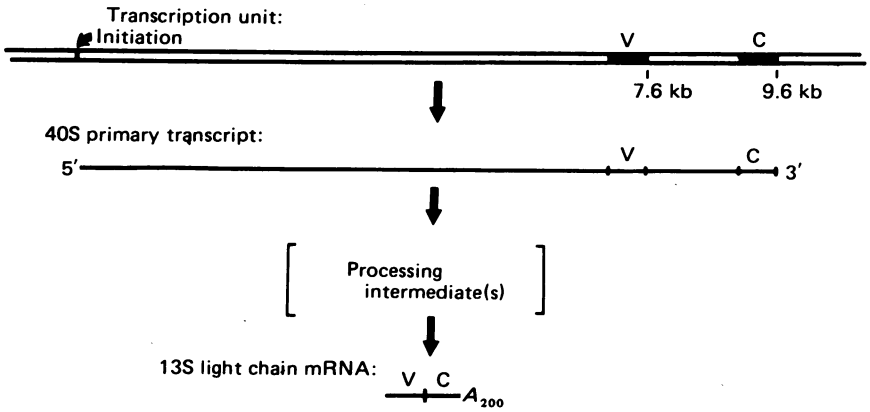

FIG. 5. The production of κ light chain mRNA from a 10-kb transcription unit in which variable and constant regions are separated. The model proposes that the joining of variable and constant regions occurs in the post-transcriptional processing of a 10-kb primary transcript. Further details are presented in the *Discussion*.

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