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Genetics

Avian sarcoma virus 17 carries the *jun* oncogene

(retrovirus/avian sarcoma/transformation/nucleotide sequence)

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ABSTRACT **Biologically active molecular clones of avian sarcoma virus 17 (ASV 17) contain a replication-defective proviral genome of 3.5 kilobases (kb). The genome retains partial *gag* and *env* sequences, which flank a cell-derived putative oncogene of 0.93 kb, termed *jun*. The *jun* gene lacks preserved coding domains of tyrosine-specific protein kinases. It also shows no significant nucleic acid homology with other known oncogenes. The probable transformation-specific protein in ASV 17-transformed cells is a 55-kDa *gag–jun* fusion product.**

Avian sarcoma virus 17 (ASV 17) was isolated from a spontaneous sarcoma in an adult chicken (1). It is a retrovirus that induces progressively growing fibrosarcomas in chickens and transforms cultured chicken embryo fibroblasts (CEF) into elongated refractile neoplastic cells. ASV 17 has a small genome and lacks sequences homologous to the avian retroviral DNA polymerase gene, *pol*. It is defective in replication. A fraction of the agar colonies induced at low multiplicities of infection of CEF are nonproducers: They are neoplastically transformed but do not release infectious ASV 17 virus. Superinfection of such nonproducer cultures with an avian leukosis helper virus generates ASV 17 pseudotypes. ASV 17 thus shows the two indicators for the presence of an oncogene in its genome: transforming activity in cell culture and defectiveness in replication. Dot-blot hybridization of ASV 17 RNA does not reveal homology with DNA probes representing standard oncogenes, including erb*A*, *erbB*, *fps*, *myb*, *myc*, *src*, and *yes* (1). Therefore, it appears worthwhile to study the genome of ASV 17 in greater detail. In the present communication we describe molecular clones and the genetic map of the ASV 17 genome, define its cell-derived oncogene, and report the sequence of this oncogene.

MATERIALS AND METHODS

Cells and Viruses. CEF cultures were prepared from 10-day-old embryos of the H&N White Leghorn line (H&N Farms, Redmont, WA) according to published techniques (2). Growth medium consisted of medium F10 with 5% calf serum and 2% 0.33 M $NaHCO_3$. The origin of ASV 17 has been described (1).

DNA and RNA. Eukaryotic DNAs were prepared according to the method described by Blin and Stafford (3). Bacteriophage and plasmid DNAs were obtained by the technique described by Maniatis *et al.* (4).

Construction of Chicken Genomic Library and Screening. DNA extracted from ASV 17-infected CEF was partially digested with restriction endonuclease *Sau*3A1, dephosphorylated with calf intestinal alkaline phosphatase, cloned in the *Bam*HI site of the λ phage vector EMBL3 (5), and packaged and propagated in the K802 strain of *Escherichia coli* by standard procedures. The complete genomic library was screened by plaque hybridization (6) using ^{32}P-labeled long terminal repeat (LTR) or *pol*-specific probes of avian retroviruses. The LTR- and *pol*-specific DNA fragments were prepared from pEcoR1D and pHindIII Bam plasmids, respectively. These plasmids were gifts from J. M. Bishop and Nancy Quintrell (University of California, San Francisco). All enzymes employed were purchased from Boehringer Mannheim and were used under the conditions recommended by the supplier.

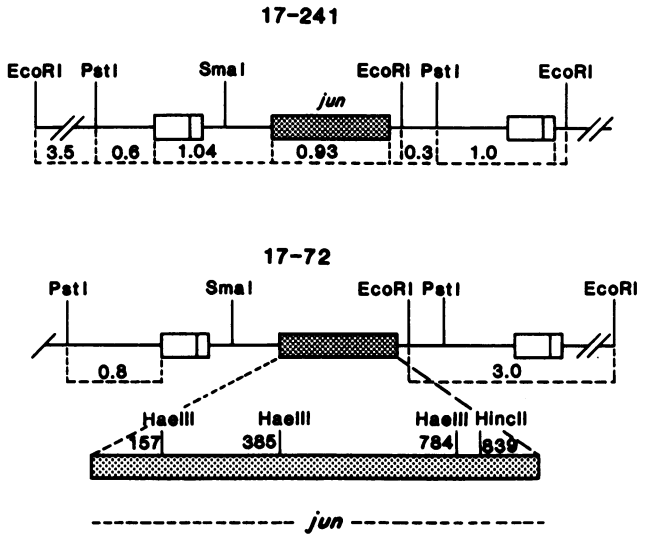

FIG. 1. Physical map of ASV 17 clones 72 and 241. The two divided rectangles in each map indicate the LTR sequences. Distances are noted in kilobases (kb) for 17-241 and 17-72. For *jun*, restriction enzyme sites are marked by nucleotide numbers, starting at the 5′ end of the insert.

Transfection. Transfection experiments were performed on CEF with recombinant λ phage DNAs without the addition of helper virus DNA, according to the method described by Kawai and Nishizawa (7).

DNA Sequencing. The DNA fragment encompassing the ASV 17 oncogene was inserted into single-stranded DNA phages M13mp19 and mp18. Deletion clones were constructed by using BAL-31 nuclease (8). Both strands were sequenced by the dideoxy chain termination method, using the Klenow fragment of DNA polymerase I (9). Ambiguous regions containing a preponderance of G and C residues were sequenced with reverse transcriptase (10).

Blot Hybridization Analysis of DNA. DNAs (20 μg per lane) were digested with restriction endonuclease *Eco*RI, fractionated by electrophoresis through a 0.8% agarose gel, detected by ethidium bromide fluorescence, and hybridized after

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Abbreviations: ASV 17, avian sarcoma virus 17; CEF, chicken embryo fibroblasts; LTR, long terminal repeat.

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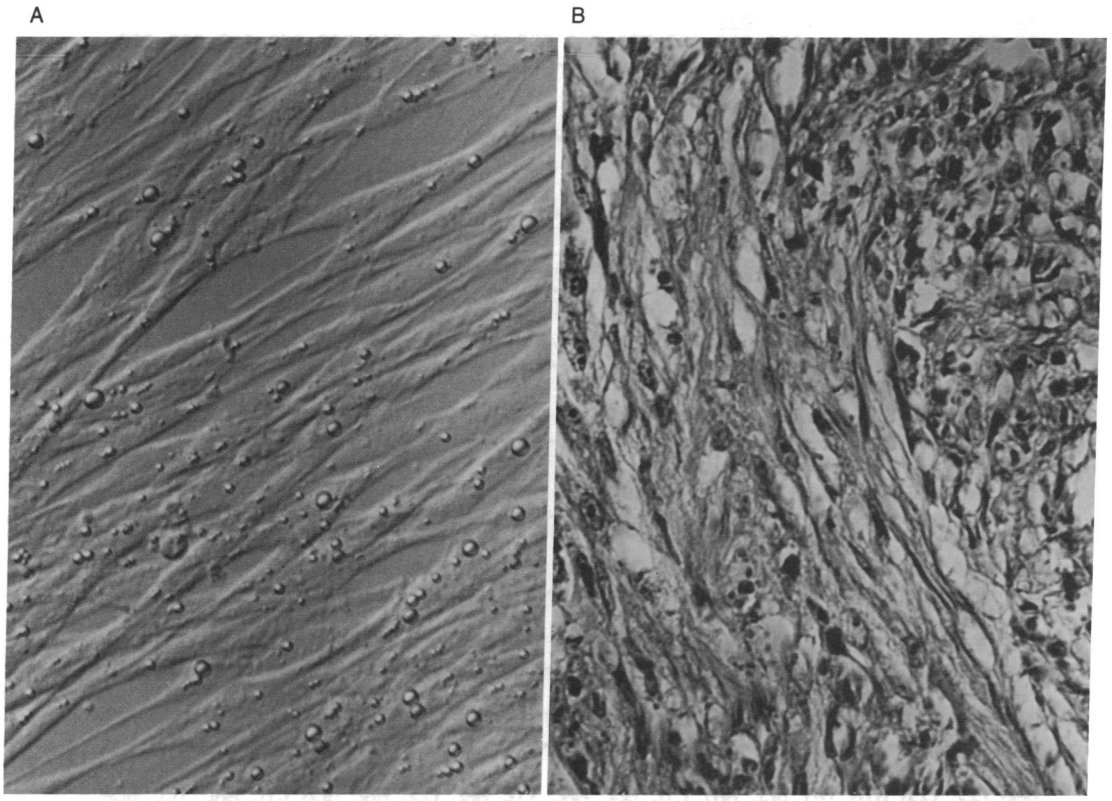

FIG. 2. (*A*) CEF transformed with transfected DNA of clone 17-241. (Interference contrast, ×250.) (*B*) Histological section of a sarcoma induced by 17-241 virus that was rescued with Rous-associated virus 1 (RAV-1, an avian leukosis virus) from nonproducing transfected CEF. (Hematoxylin/eosin stain, ×250.)

transfer to a nitrocellulose filter (11). Southern blot hybridization was performed at 42°C for 36 hr in 50% (vol/vol) formamide/5× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate)/5× Denhardt's solution (1× Denhardt's solution = 0.02% each of Ficoll, polyvinylpyrrolidone, and bovine serum albumin)/0.1% $NaDodSO_4$ containing dena-

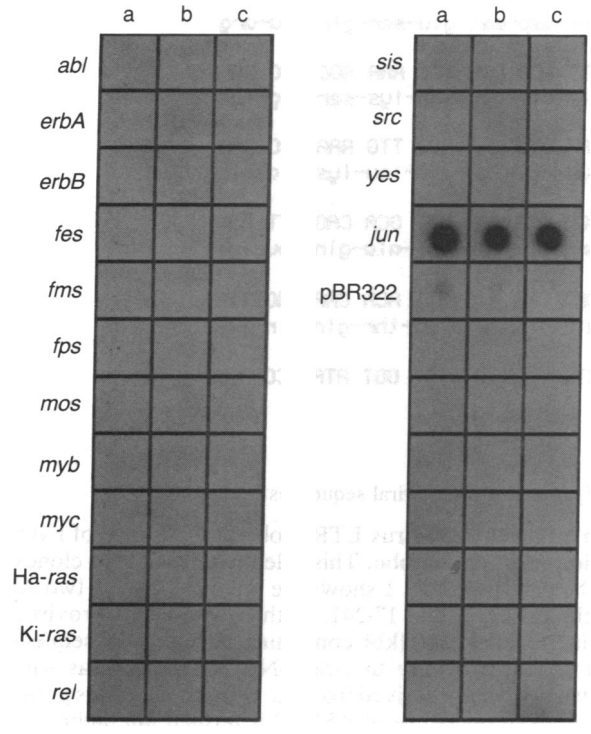

FIG. 3. Dot-blot hybridization of DNA from several oncogenes and *jun*. DNA from each oncogene was spotted and hybridized to a *jun*-specific probe. Columns: a, 1 μg of DNA; b, 0.5 μg of DNA; c, 0.25 μg of DNA. pBR322 was used as negative control.

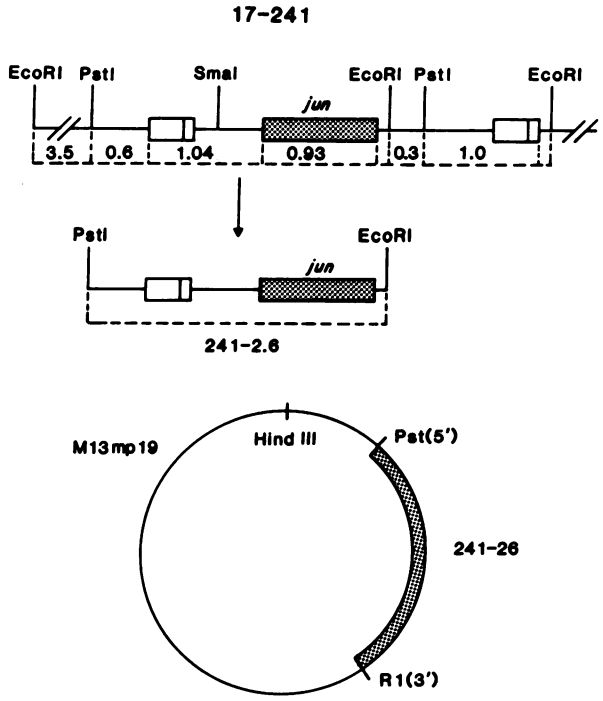

FIG. 4. Subcloning of the *jun*-containing *Pst* I–*Eco*RI fragment in M13mp19. Labeling is as in Fig. 1. DNA of clone 17-241 was digested with restriction endonucleases *Pst* I and *Eco*RI. A 2.64-kb fragment was isolated that hybridized with LTR, *gag*, and *env* probes. The fragment was inserted into M13mp19. The 5′ and 3′ deletion clones for sequencing were generated by treatment with BAL-31.

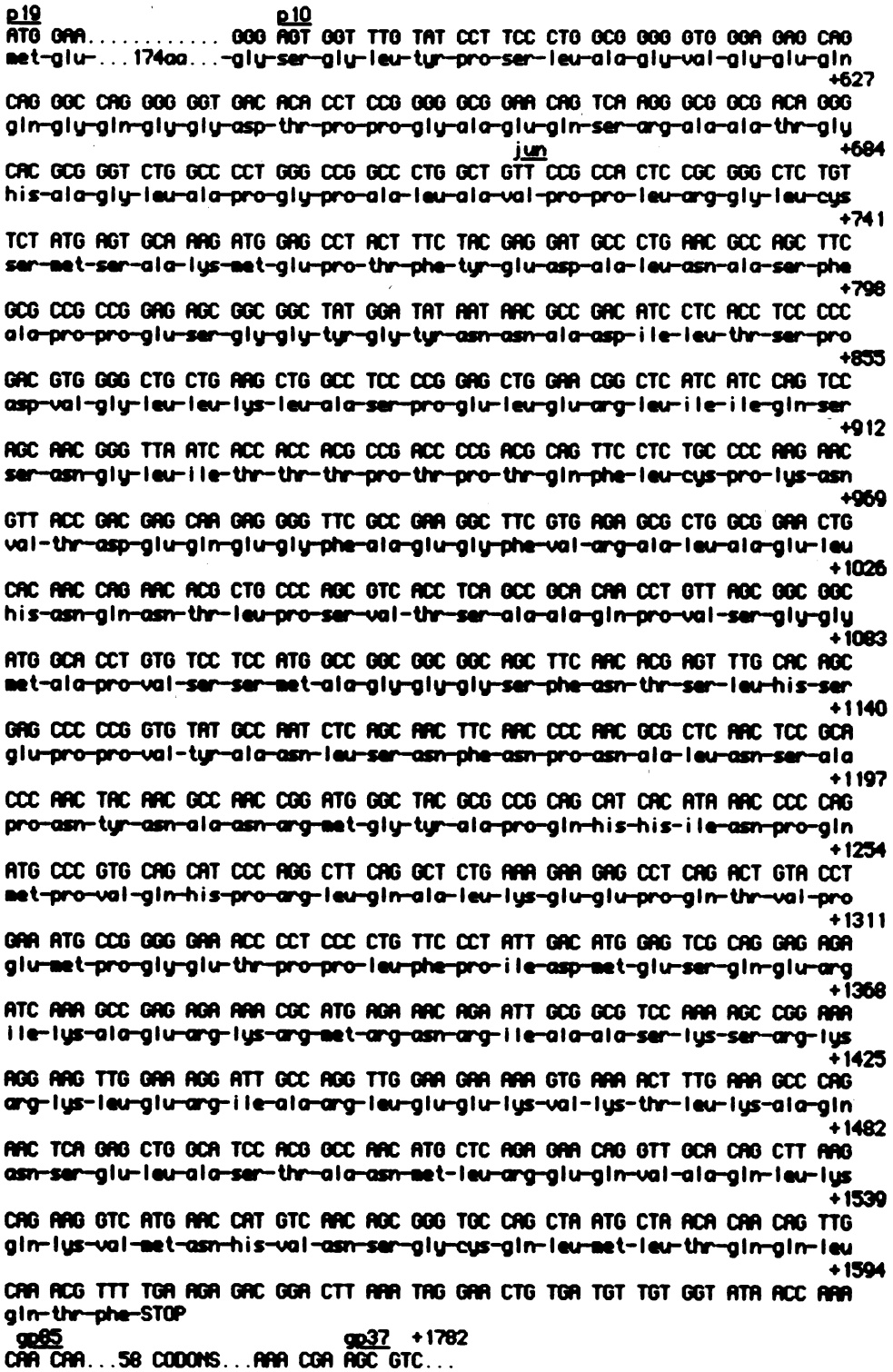

FIG. 5. Nucleotide and amino acid sequences of *jun* and adjacent viral sequences.

tured salmon sperm DNA at 100 μg/ml. Filters were washed with 2× SSC/0.1% $NaDodSO_4$ then with 0.1× SSC/0.1% $NaDodSO_4$ and processed for autoradiography. For dot hybridization, 0.25–1.0 μg of DNA was blotted onto nitrocellulose filters by employing a manifold apparatus (Schleicher & Schuell) and fixed by baking at 80°C in a vacuum oven. The DNA filters were hybridized at 42°C for 20 hr, washed, and processed as described above.

RESULTS

Cloning of ASV 17. Genomic clones of the ASV 17 provirus in the phage vector EMBL3 were selected by hybridization with an avian retrovirus LTR probe and absence of hybridization with a *pol* probe. This selection eliminated clones of the helper virus. Fig. 1 shows the physical map of two ASV 17 clones: 17-72 and 17-241. Both encompass a provirus of about 3.5 kilobases (kb) containing an insert of sequences that do not hybridize to viral DNA probes and, as will be shown below, are derived from the cell genome. This putative cell-derived oncogene of ASV 17 is termed *jun*, abbreviated from Japanese *ju-nana*, the number 17. The two LTRs of the provirus flank partial *gag* and partial *env* sequences. Both clones 17-72 and 17-241 are biologically active. Transfection of the cloned DNA into CEF results in the formation of

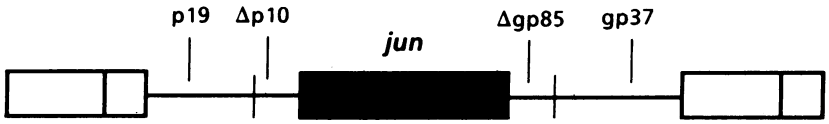

FIG. 6. Map of the ASV 17 genome. Divided rectangles indicate LTR sequences.

transformed cell foci that show the characteristic morphology of ASV 17 infection: fusiform refractile cells growing in parallel orientation and in multiple layers. These transformed cells do not release infectious virus, and they can be subcultured, continuing to grow for 25–30 cell generations (Fig. 2*A*). Superinfection with a helper virus leads to the rescue of infectious ASV 17. The rescued cloned virus is able to induce foci in CEF and fibrosarcomas in chickens (Fig. 2*B*). The clones were also tested for homology with several known oncogenes by dot-blot hybridization. No homology was found with *erbA, erbB, myc, myb, fps, rel, yes, src*, Ha-*ras*, Ki-*ras*, *fes*, *fms*, or *sis* by using a ^{32}P-labeled probe from clone 17-241 (Fig. 3). Homology to *mos* and *abl* also was not found (data not shown).

DNA Sequence of *jun*. The 2.6-kb restriction fragment (241-2.6) delineated by the *Pst* I site to the left of the provirus and the *Eco*RI site to the right of *jun* was subcloned in M13mp19 phage (Fig. 4). To facilitate sequencing of the 241-2.6 subclone containing the *jun* insert as well as flanking viral sequences, deletions from the 5′ and 3′ end of the 241-2.6 insert were generated with the nuclease BAL-31. The 5′ deletion clones were subcloned in M13mp18 and the 3′ deletion clones in M13mp19. These deletion clones were then used to sequence both strands of the 241-2.6 fragment. Fig. 5 presents the sequence of *jun* and its flanking viral contexts. ASV 17 contains the 5′-terminal portion of the *gag* gene, encompassing all of p19 and about 70% of p10 (Fig. 6). *jun* has a length of 935 nucleotides. The 3′ flanking sequences of *jun* consist of the 3′ 18% of the gp85 and all of the gp37 coding sequences (Fig. 6). An open reading frame extends from the initiator codon of p19 to near the end of *jun*. It can code for a *gag*-linked transformation-specific 55-kDa protein. We have detected this putative *jun* product in ASV 17-transformed cells (W. Uckert, T.J.B., and P.K.V., unpublished observations).

Cellular Origin of *jun*. One of the subclones generated by BAL-31 digestion, clone 241-10-2, contained most of *jun* but none of the retroviral sequences. It was used to prepare a *jun*-specific DNA probe. With this probe, DNA from several vertebrate species was screened for relatedness to *jun*. Homology was found between *jun* and DNA of chicken and Japanese quail (Fig. 7) and mouse, rat, and human (data not shown).

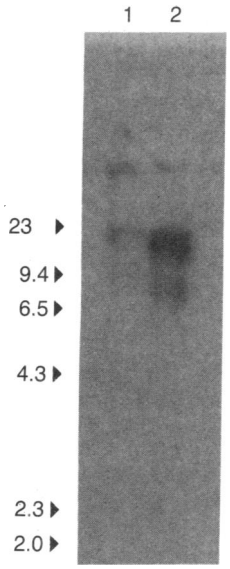

FIG. 7. Southern hybridization of vertebrate genomic DNA to *jun*. Twenty micrograms of each DNA was digested with *Eco*RI before electrophoresis, Southern transfer, and hybridization to a *jun*-specific probe. Lanes: 1, DNA from CEF; 2, DNA from a Japanese quail continuous cell line (QT35). Size markers, in kilobase pairs, were generated by digestion of λ phage DNA with *Hin*dIII.

DISCUSSION

jun is a cell-derived retroviral insert that has not been described previously, to our knowledge. Like all other cellular inserts of retroviruses that transform cells in culture and, within a short latent period, induce tumors in the animal, *jun* probably functions as a determinant of oncogenesis. We tentatively consider *jun* the oncogene of ASV 17, pending formal proof of its carcinogenic activity. *jun* does not show close nucleic acid sequence homology to any known oncogene (see also ref. 12). It does not contain conserved domains that are characteristic of genes coding for tyrosine-specific kinases. The putative transformation-specific protein p55$^{gag\text{-}jun}$ lacks kinase activity, and ASV 17-transformed cells do not show increased levels of cellular phosphotyrosine (W. Uckert, M. Kamps, T.J.B., and P.K.V., unpublished observations). Besides ASV 17, we know of no avian sarcoma virus with an oncogene that does not code for a tyrosine-specific kinase.

The putative ASV 17 transformation-specific protein p55$^{gag\text{-}jun}$ is presumably derived from a messenger RNA in which gag and *jun* information are fused, using the gag reading frame and continuing into *jun*. The other two reading frames contain numerous stop codons in the *jun* sequence. These also make unlikely the existence of a *jun–env* fusion protein. p55$^{gag\text{-}jun}$ contains the proteolytic cleavage site between p19 and p10. In ASV 17-infected cells that are also infected by helper virus, p55$^{gag\text{-}jun}$ could therefore be proteolytically processed to a p36$^{gag\text{-}jun}$ and p19^{gag}. Whether the partial *env* sequences of the ASV 17 genome are expressed is not known.

jun sequences appear to have been acquired by ASV 17 from a vertebrate genome, probably that of the chicken. Preliminary observations suggest that *jun* also occurs in other recent avian sarcoma virus isolates. Whether these represent independent recombination events between an avian retrovirus and proto-*jun* or a horizontal spread of ASV 17 remains to be determined.

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