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Correct splicing of a chicken ovalbumin gene transcript in mouse L cells

(eukaryotic gene/polyadenylylation/transformation/transcription)

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ABSTRACT **Mouse thymidine-kinase-negative L cells were transformed with a cloned chicken ovalbumin gene linked to the cloned thymidine kinase gene from herpes simplex virus type 1. Most thymidine-kinase-positive clones contained one or more copies of the ovalbumin gene, which were stably maintained for at least 6 months during continuous culture under selective conditions. Transcription of the ovalbumin gene was detected at a low level, producing RNA molecules that were correctly spliced and polyadenylylated by comparison with genuine ovalbumin mRNA. However, the 5′ end of these RNA molecules does not correspond to that of ovalbumin mRNA.**

Molecular cloning has made possible the determination of the structure of the chicken ovalbumin gene (1), which is expressed under hormonal control in the oviduct tubular gland cells (2). By comparison with the structure of other genes, we have identified several conserved sequences that may play a role in the regulation of the expression of the ovalbumin gene and in the splicing of its primary transcript. However, for the true significance of these sequences to be evaluated, they, or mutants derived from them, must be tested in appropriate *in vitro* or *in vivo* systems (3, 4). We describe here our first approach to the development of such an *in vivo* biological system, which consists of introducing the ovalbumin gene into a heterologous cultured eukaryotic cell. Previous work (5–7) has indeed shown that it is possible to introduce foreign DNA into thymidine-kinase-negative (TK^-) mouse L cells by cotransformation with a thymidine kinase gene from herpes simplex virus type 1. Using this approach, we have introduced the chicken ovalbumin gene into L cells.

MATERIALS AND METHODS

Plasmids and Enzymes. pBov1 and pBov2 were constructed as described in the legends to Figs. 1*a* and 3*b* and in the text. Other plasmids and enzymes have been described elsewhere (8–12). Ovalbumin mRNA (ov-mRNA) was a gift from M. LeMeur and A. Krust.

Cell Culture and Transformation. $LMTK^-$ cells were grown in monolayer cultures as described (5). $LMTK^+$ cells were maintained in the presence of hypoxanthine/aminopterin/thymidine medium. Six micrograms of pBov1 and 1 μg of TK-M4 plasmid DNA (7, 8, 13) were cleaved at their single *Sal* I sites, extracted with phenol, and precipitated with ethanol. They were then ligated with Biolabs T4 DNA ligase (1 μl) in a volume of 50 μl for 3 hr before phenol extraction and ethanol precipitation. The heterologous concatenates were used to transform $LMTK^-$ cells on five 100-mm petri dishes as described (5). The TK^+ phenotype was selected as described (5), and 10 isolated colonies of cells were picked by using the sterile cylinder technique.

Analysis of DNA. DNA was isolated from confluent cultures. After digestion with proteinase K (100 μg/ml), phenol/chloroform extraction, and ethanol precipitation, the DNA was digested with a restriction enzyme by using adenovirus DNA as digestion marker (10). Electrophoresis, blotting, and hybridization to nick-translation probes were as described (10).

Analysis of RNA. Total RNA was isolated from confluent cells by the hot phenol extraction technique (14). Poly(A)-RNA and RNA not containing poly(A) were separated by two cycles of chromatography on oligo(dT)-cellulose. For hybridization, 50 ng of 5′-end-labeled DNA and the RNA samples were dissolved in 50 μl of 10 mM 1,4-piperazinediethane sulfonate (Pipes), pH 6.4/0.4 M NaCl/80% formamide and incubated for 48 hr at 46.5° or 40°C after treatment for 5 min at 85°C. Five hundred microliters of ice-cold S1 mixture (15) containing S1 nuclease was then added and incubation was carried out for 1 hr at 37°C. Samples were adjusted to 200 mM Tris·HCl, pH 7.5/20 mM EDTA, extracted with phenol/chloroform, and precipitated with ethanol before analysis on agarose gels or 8% acrylamide/7 M urea thin gels (16). Autoradiography was as described (11).

DNA Labeling. DNA nick-translation and 5′-end-labeling with [γ-^{32}P]ATP and T4 polynucleotide kinase were as described (10, 11). pBov2 was linearized with *Xba* I or *Bgl* II, 5′-end-labeled, and redigested with *Hin*dIII or *Bam*HI (Fig. 3*b*). Singly end-labeled fragments were then isolated on 5–20% sucrose gradients. The *Ava* II–*Pst*4 fragment of the ovalbumin gene (Fig. 1*a*), 5′-end-labeled at the *Ava* II site, was prepared from a 5′-end-labeled *Ava* II digest of the 3.3-kilobase (kb) *Eco*RI fragment of the gene (Fig. 1*a*) by redigestion with *Pst* I and electrophoresis on an acrylamide gel (11).

Biohazards associated with the experiments described here were examined by the French National Control Committee.

RESULTS

Isolation of Mouse L Cell Clones Containing the Ovalbumin Gene. The 12.5-kb *Hha* I fragment from the cloned ovalbumin gene in λC4-ov5 (1), which contains all the mRNA-coding sequences (17), was cloned in the *Hin*dIII site of pBR322. This recombinant, pBov1 (Fig. 1*a*), was linearized by digestion with *Sal* I restriction endonuclease and ligated with a *Sal* I-linearized plasmid containing a *Kpn* I fragment of the herpes simplex virus *TK* gene (TK-M4; refs. 8 and 13) to form concatenates that were used to transform $LMTK^-$ cells (5, 18). The TK^+ transformants were selected in hypoxanthine/aminopterin/thymidine medium, and nine of the resulting colonies

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Abbreviations: kb, kilobase; bp, base pair, TK, thymidine kinase; ov-mRNA, ovalbumin mRNA; ds cDNA, double-stranded cDNA.

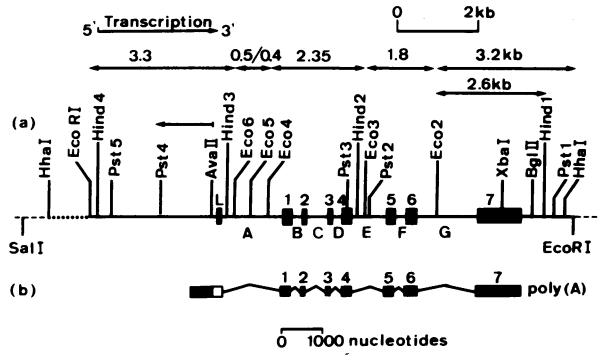

FIG. 1. (*a*) Map of the chicken ovalbumin gene plasmid pBov1. pBov1 was constructed from the *Hha* I fragment of λC4-ov5 (see text and ref. 1). pBR322 plasmid vector sequences are shown as dashed lines, λ sequences as a dotted line, and exons (L and 1–7) as heavy lines. Introns are lettered A–G. Restriction enzyme sites shown above the figure are found in the λC4-ov5 clone (1); those situated below the figure belong to the pBR322 vector. The *Hha* I sites were destroyed during insertion into pBR322. The origin of the 2.6-kb *Eco*2–*Hind*1 fragment cloned in pBov2 (Fig. 3*b*) is shown. A horizontal arrow above the figure identifies the *Pst*4–*Ava* II fragment used for S1 nuclease mapping. Only those *Ava* II, *Xba* I, and *Bgl* II sites discussed in the text are shown. (*b*) Map of the ovalbumin gene transcript present in transformed L cells. Exon transcripts (heavy lines) are numbered 1–7 as in *a*. Light lines represent sequences not present in the transcript and presumably spliced out of a nuclear precursor. The map is drawn to the same scale as in *a*. For significance of the region not blacked in, see *Discussion*.

were analyzed (clones Lov1–9). Two control clones, M25-3 and M25-4, from a parallel transformation with *Sal* I-cleaved TK-M2 plasmid alone, were also examined. DNA was isolated from these clones, digested with *Eco*RI, electrophoresed on agarose gels, transferred to nitrocellulose filters (19), and hybridized to the nick-translated ^{32}P-labeled *Hha* I fragment of pBov1 that contains the ovalbumin gene (Fig. 1*a*). DNA from seven of the nine Lov colonies responded to this probe: Lov1–4 (Fig. 2, lanes 2–5), Lov6 (not shown), Lov8 (Fig. 2, lane 8), and Lov9 (not shown). DNA from clones Lov5 (Fig. 2, lane 6) and Lov7 (not shown) or from "control" clones M25-3 (Fig. 2, lane 10) or M25-4 (not shown) showed no hybridization. Comparison of the patterns obtained from the Lov clones 1–4, 6, 8, and 9 with that of an *Eco*RI digest of pBov1 (Fig. 2, lane 7) indicated that all of the *Eco*RI ovalbumin gene fragments (Fig. 1*a*) were present in the *Eco*RI digests of DNA from the transformed Lov

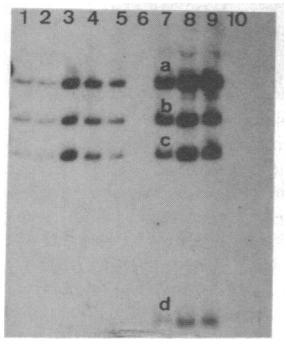

FIG. 2. Analysis of DNA from transformed L cell clones. DNA (20 μg) from the clones was digested with *Eco*RI, electrophoresed on a 1.5% agarose gel, transferred to a nitrocellulose filter, and hybridized to a nick-translated ovalbumin gene probe derived from pBov1 (see text). Lanes 2–6, 8, and 10, DNA from clones Lov1–5, Lov8, and M25-3, respectively. Lane 7, *Eco*RI digest of pBov1. Size of the bands (in kb): a, 3.2–3.3; b, 2.35; c, 1.8; d, 0.4–0.5. (See Fig. 1*a* for the origin of these bands.) Lanes 1 and 9, DNA from clones Lov1 and Lov8, respectively, isolated after 6 months in continuous culture.

clones. Similar results (not shown) were obtained with *Hin*dIII or *Pst* I (Fig. 1*a*). The ovalbumin gene copies have thus not undergone detectable rearrangement during the transformation process. From the intensities of bands in experiments such as that shown in Fig. 2 relative to those obtained from known amounts of ovalbumin gene sequences, we estimate a wide range of copy numbers from about 1 in Lov1 to more than 30 in Lov8.

The stability of both ovalbumin gene structure and gene copy numbers was investigated after 6 months of continuous culture. Comparison of lanes 1 and 2 (Lov1) or 8 and 9 (Lov8) in Fig. 2 indicates that both copy number and gene arrangement have remained unchanged over the 6 months during which this work was done.

Presence of a Spliced Polyadenylylated Transcript of the Ovalbumin Gene in L Cell Ovalbumin Clones. Total RNA was isolated from the L cell ovalbumin clones and analyzed by a modification (20) of the S1 nuclease mapping method of Berk and Sharp (15) by using pCR1ov2.1, a double-stranded (ds) cDNA plasmid that contains an almost full-length copy of ov-mRNA, lacking only the 14 nucleotides corresponding to the 5′ end of ov-mRNA (9, 17). The plasmid was linearized with *Xba* I (Fig. 3*a*), 5′-end-labeled and hybridized to total RNA from clones Lov1, 4, 6, and 8 under conditions where DNA·DNA hybridization was minimized (21). After S1 nuclease treatment, the resulting hybrids were electrophoresed on agarose gels (Fig. 4*A*). Bands of identical size [1300 base pairs (bp), arrowheads] are seen with RNA from Lov clones 8, 1, 4, and 6 (lanes 4–7) and with ov-mRNA (lanes 2 and 3). This is exactly the result expected if the Lov clones contain transcripts of the DNA (−)strand coding for the ovalbumin gene capable of protecting from S1 nuclease digestion the entire length of the ovalbumin ds cDNA sequences from its *Xba* I site to its 5′ end (Fig. 3*a*). No band was detected (not shown, but see below) that could have corresponded to transcription from the ovalbumin

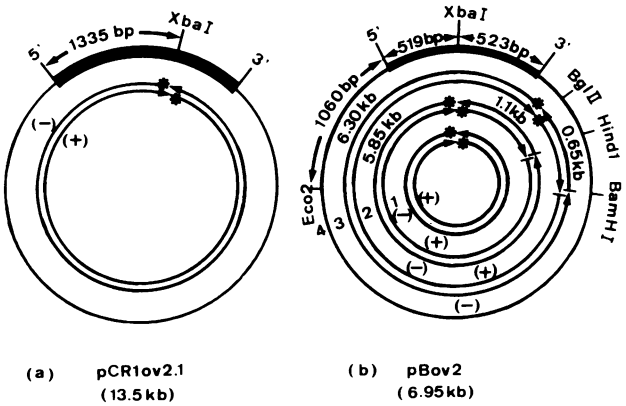

FIG. 3. (*a*) Map of the ovalbumin ds cDNA plasmid pCR1ov2.1 (outer circle, see refs. 9 and 17). The ds cDNA sequences are shown as a heavy line. The *Xba* I site used for S1 mapping is shown; 5′ and 3′ correspond to the 5′ and 3′ ends of the inserted ds cDNA. Asterisks represent the ^{32}P label at the 5′ ends of the *Xba* I site (inner circle). Counterclockwise arrow corresponds to the ovalbumin-coding DNA (−) strand; clockwise arrow corresponds to the noncoding DNA (+) strand. bp, Base pairs. (*b*) Map of plasmid pBov2. The 2.6-kb *Eco*2–*Hind*1 fragment containing exon 7 (heavy line, see Fig. 1*a*) was cloned in the corresponding sites of pBR322 (circle 4). The *Xba* I, *Bgl* II, and *Hind*1 sites of the chicken ovalbumin DNA region (Fig. 1*a*) and the pBR322 *Bam*HI site used for S1 mapping are shown. 5′ and 3′ correspond to the 5′ and 3′ ends of exon 7. G represents the 3′ moiety of intron G (see Fig. 1*a*). Various fragments used for S1 mapping (see text) are represented (circles 1–3). Asterisks represent the ^{32}P label at the 5′ ends of *Xba* I and *Bgl* II sites. Other symbols are as defined in *a*.

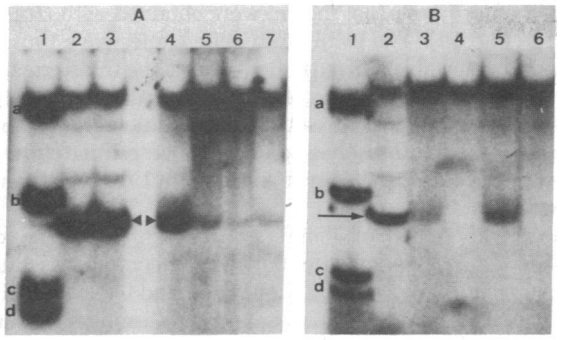

FIG. 4. (*A*) Analysis of RNA from clones Lov1, 4, 6, and 8. Total RNA of Lov clones (lanes 4–7) or purified ov-mRNA (lanes 2 and 3) was hybridized to pCR1ov2.1 (5′-end-labeled at its *Xba* I site; Fig. 3*a* and text). The hybrid was treated with S1 nuclease and electrophoresed on a 2.5% agarose gel. Lane 1: bands a–c, size markers from an *Eco*RI/*Bgl* II/*Xba* I digest of pBov2 (Fig. 3*b*); band d, marker from a *Mbo* II digest of the 2.6-kb *Eco*2–*Hin*d1 fragment of the ovalbumin gene (Fig. 1*a*). Sizes of the bands (in kb): a, 4.6; b, 1.5; c, 0.86; d, 0.83. Lanes 4–7: RNA from clones Lov8 (100 μg), Lov1 (50 μg), Lov4 (50 μg), and Lov6 (50 μg), respectively. Bands at the top of the gel represent renatured pCR1ov2.1 DNA. Arrowheads indicate the position of the 1.3-kb band discussed in the text. (*B*) Polyadenylylation of ovalbumin gene transcript in transformed L cells. RNA (100 μg) from clones Lov1 and Lov8 was separated into poly(A)-RNA and RNA not containing poly(A) before hybridization to pCR1ov2.1 (5′-end-labeled at its *Xba* I site) and S1 treatment as above. Lane 1, size markers as in *A*. Lane 2, ov-mRNA hybridized in parallel, 0.2 ng. Lanes 3 and 5, poly(A)-RNA from clones Lov1 and Lov8, respectively. Lanes 4 and 6, RNA not containing poly(A) from clones Lov1 and Lov8, respectively. Bands at the top of the gel represent renatured pCR1ov2.1 DNA. Arrow indicates the position of the 1.3-kb band.

nonsense DNA (+)strand (Fig. 3*a*). Similar results were obtained with RNA from Lov2, 3, and 9 clones, but no 1300-bp band was seen when the RNA of the "control" clones was analyzed (not shown). From these results it appears that the Lov clones contain a processed ovalbumin RNA transcript from which the transcripts of introns B–G (and possibly A) have been spliced out. The transcript contains most, if not all, of the sequence between the 5′ end of ov-mRNA and the *Xba* I site present in exon 7 (Figs. 1*a* and 3*a*).

The results shown in Fig. 4*B* indicate that the ovalbumin transcript present in Lov cells is polyadenylylated. Poly(A)-RNA and RNA without poly(A), fractionated by oligo(dT)-cellulose chromatography, from clones Lov1 and Lov8 were analyzed as above. The bulk of the transcripts capable of protecting the probe from S1 digestion to yield the 1300-bp band (arrow) occurred in the poly(A)-RNA fraction (lanes 3 and 5, for Lov1 and Lov8 clones, respectively), whereas no RNA transcripts were detected in the RNA fraction without poly(A) (lanes 4 and 6).

The 3′ End of the Ovalbumin RNA Transcripts Found in Transformed L Cell Clones Corresponds to That of ov-mRNA. The above studies suggest that at least part of the ovalbumin exon 7 is transcribed in the Lov cells and that transcripts of introns B–G are correctly spliced out. To further support the conclusion that intron G transcript is spliced out in the Lov cell and to determine whether this transcript has the same 3′ end as ov-mRNA, we used as probe plasmid pBov2 (Fig. 3*b*), which contains the 2.6-kb *Eco*2–*Hin*d1 fragment of the ovalbumin gene (Fig. 1*a*) inserted in the corresponding sites in pBR322. The *Xba* I-linearized plasmid was 5′-end-labeled (Fig. 3*b*, circle 1) and hybridized with total Lov RNA. After S1 nuclease treatment, the resulting hybrids were electrophoresed on agarose gels. If the Lov cell transcripts of exon 7 terminate at the same site as ov-mRNA and have the intron G transcript spliced out, then the transcripts would be complementary to pBov2 over the length of exon 7 only (1042 bp, see ref. 17). The initial product of S1 digestion would then be a 1042-bp DNA·RNA hybrid containing a single nick on the DNA strand at the *Xba* I site. Further S1 treatment will cut at this nick to yield a 519-bp (see ref. 17) hybrid corresponding to the distance between the *Xba* I site and the 5′ end of exon 7 (see Fig. 3*b*). On the other hand, if the Lov exon 7 transcripts are linked to intron G transcript sequences, the corresponding hybrids would be longer (up to maxima of 2100 bp and 1580 bp, respectively; see Figs. 1*a* and 3*b*).

The result of such an experiment using total RNA from Lov8, 6, and 4 clones is shown in Fig. 5*A*, lanes 4–6. These lanes may be compared to lanes 2 and 3, in which the hybridization was carried out with ov-mRNA. In both cases, a band at 500 bp is found (lower arrowhead). In addition, in lanes 4 (Lov8) and 2 (ov-mRNA) a second band is seen at 1050 bp (upper arrowhead). This band is also visible on the original autoradiogram in lanes 3, 5, and 6. (The intensity of this band was variable and not directly related to the amount of ovalbumin RNA sequences; it represents an intermediate stage in the S1 nuclease digestion whose kinetics were affected by the amount of total RNA present in the incubation mixture.) Similar results were found with RNA from Lov1, 2, and 3 clones, but no bands, at either 500 or 1050 bp, were detected with "control" M25-3 RNA (lane 7).

The probe used, pBov2 cut with *Xba* I and 5′-end-labeled, is labeled on both the (−) and (+) strands of the DNA [Fig. 3*b*, circle 1, where (−)strand codes for ov-mRNA] and could therefore hybridize and give a signal with transcripts of the ov-mRNA-coding and -noncoding strands. To rule out the possibility that the bands observed in Fig. 5*A* result from the

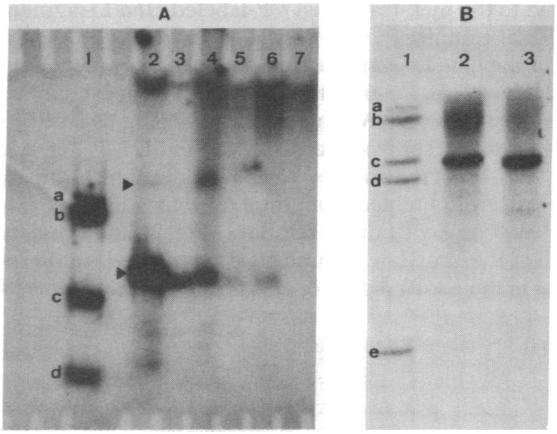

FIG. 5. Analysis of 3′ end of ovalbumin RNA transcripts present in clones Lov4, 6, and 8. (*A*) Total RNA from Lov clones or ov-mRNA (1 ng) was hybridized to pBov2 (5′-end-labeled at the Xba I site; Fig. 3*b*, circle 1). The mixture was treated with S1 and hybrids were electrophoresed on a 2.5% agarose gel. Lane 1: size markers from a *Mbo* II digest of the 2.6-kb *Eco*2–*Hin*d1 fragment of the ovalbumin gene (Fig. 1*a*). Size of the bands (in kb): a, 0.86; b, 0.83; c, 0.48; d, 0.25. Lanes 2 and 3: 0.9 and 0.1 ng of ov-mRNA, respectively. Lanes 4–7: RNA from clones Lov8 (80 μg), Lov6 (20 μg), Lov4 (50 μg), and control clone M25-3 (100 μg), respectively. The 1050-bp and 500-bp fragments discussed in the text are marked by arrowheads. Bands at the top of the gel represent renatured pBov2 DNA. (*B*) Poly(A)-RNA from clones Lov1 and Lov8 was hybridized to a 5.85-kb *Xba* I–*Hin*d1 fragment of pBov2 (Fig. 3*b*, circle 2) 5′-end-labeled at the *Xba* I site only [(−)strand]. After S1 treatment, the material was electrophoresed on an 8% acrylamide/7 M urea gel. Lane 1: size markers from a *Hin*dIII digest of simian virus 40 DNA. Size of bands (in kb): a, 1.77; b, 1.17; c, 0.52; d, 0.45; e, 0.21. Lanes 2 and 3: poly(A)-RNA from clones Lov1 and Lov8, respectively.

transcription of the DNA (+)strand, we repeated the above experiment using as probe a 5.85-kb *Xba* I-*Hin*dI fragment of pBov2 labeled only on the (−)strand at the *Xba* I site (see Fig. 3*b*, circle 2, and *Materials and Methods*) and thus capable of detecting transcripts of the ov-mRNA-coding DNA (−)strand only. When this probe was used for S1 mapping with poly(A)-RNA from Lov1 and 8 cells, the 500-bp band was again detected (Fig. 5*B*, lanes 2 and 3). On the other hand, no bands were found when the 1.1-kb probe corresponding to the (+)-strand was used (not shown). These results, in agreement with those obtained above using the probe derived from plasmid pCR1ov2.1, demonstrate that the ovalbumin gene transcripts in Lov cells are read from the same strand as is ov-mRNA in chicken oviduct cells.

To confirm that the 3′ end of the Lov clone transcript maps in the same region as the 3′ end of ov-mRNA, an S1 mapping experiment was carried out with DNA fragments labeled at the *Bgl* II site of the ovalbumin gene lying about 300 bp downstream from the sequences coding for the 3′ terminus of ov-mRNA (22) (Figs. 1*a* and 3*b*, circle 3). Plasmid pBov2 (Fig. 3*b*) was linearized with *Bgl* II, 5′-end-labeled, and digested with *Bam*HI. The 6.30-kb fragment labeled on the DNA (−)strand (Fig. 3*b*, circle 3) and the 0.65-kb fragment labeled on the DNA (+)strand were isolated, hybridized to poly(A)-RNA from Lov1 and 8 cells, treated with S1 nuclease, and electrophoresed. No bands were detected (results not shown) with the 6.35-kb fragment specific for the (−)strand, as expected if the 3′ termini of the Lov clone transcripts and ov-mRNA map in the same region. However, transcripts corresponding to the *Bgl* II–*Hin*dI region of the DNA (+)strand are present (band b, Fig. 6*A*, lanes 2 and 3; band a is derived from renatured probe). We have not yet found any evidence for transcription of this region in hen oviduct, but transcripts from the *Bgl* II–*Hin*dI region were detected in the RNA synthesized *in vitro* by oviduct nuclei (R. Palmiter, M. LeMeur, and P. Chambon, unpublished results).

Ovalbumin RNA Present in Transformed L Cells Is Longer than ov-mRNA by about 650 Nucleotides Located at Its 5′ End. RNA from Lov cells was analyzed by electrophoresis of the RNA on methylmercury hydroxide gels (23), transfer to diazobenzyloxymethyl-paper (24), and hybridization with a nick-translated ^{32}P-labeled ds cDNA probe, Hhaov (10), derived from pCRov2.1 (9). The results (Fig. 6*B*) show a major band in Lov8, 1, and 4 clones (lanes 2–4, respectively), which correspond to a transcript about 2500 nucleotides long. This transcript is 650 ± 150 nucleotides longer than ov-mRNA, which is about 1900 nucleotides (lane 5).

The extra 650 nucleotides are transcribed from the DNA region located upstream from the sequence coding for the 5′ end of the ov-mRNA. This was shown by using the *Ava* II–*Pst*4 fragment (Fig. 1*a*) as a probe for S1 mapping. Poly(A)-RNA from Lov1 and 8 cells was hybridized with an *Ava* II–*Pst*4 fragment labeled at the *Ava* II 5′ end only [(−)strand]; the result of the autoradiography after S1 nuclease treatment and electrophoresis is shown in Fig. 6*C*. Both Lov1 (lane 3) and Lov8 (lane 4) cells contain RNA transcripts of the DNA (−)strand, covering the *Ava* II site and extending for about 550 nucleotides beyond it, which are not present in the control clone, M25-3 (lane 5). Because the *Ava* II site is situated at about 130 bp from the sequence coding for the 5′ end of ov-mRNA (Fig. 1*a*), this last result agrees with the length measurement that indicates that the Lov cell transcript should be about 650 nucleotides longer than ov-mRNA at its 5′ end.

Finally, in a parallel experiment (not shown) with the same RNA and, as a probe, plasmid pBov2 labeled to a similar specific activity at its *Xba* I site (Fig. 3*b*, circle 1), we showed that transcripts from the 5′ and 3′ regions of the ovalbumin gene in Lov cells are present in roughly equal amounts.

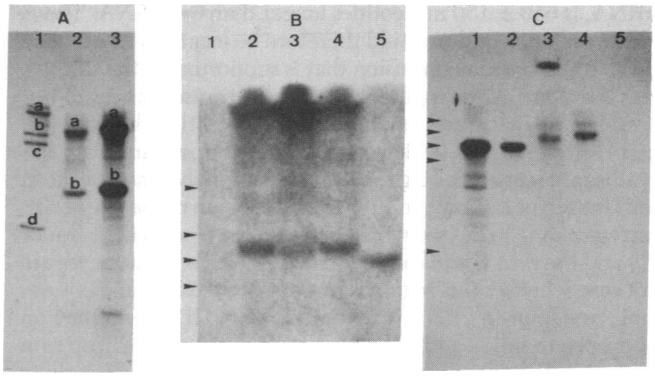

FIG. 6. Further analysis of ovalbumin RNA transcripts present in transformed cells. (*A*) Evidence for a transcript from the noncoding strand beyond the 3′ end of the ov-mRNA coding sequence. Lanes 2 and 3: poly(A)-RNA from clones Lov1 and Lov8 was hybridized to a 0.65-kb *Bgl* II–*Bam*H1 fragment of pBov2 DNA (Fig. 3*b*, circle 3) which was labeled on the DNA (+)strand at the *Bgl* II site. After S1 digestion, the hybrid was electrophoresed on an 8% acrylamide/7 M urea gel. Band a derives from the renatured 0.65-kb fragment; band b corresponds to the DNA·RNA hybrid. Lane 1: size markers from a *Hin*dIII digest of simian virus 40 DNA; size of the bands (in kb): a, 1.17; b, 0.52; c, 0.45; d, 0.21. (*B*) Size analysis of ovalbumin gene transcripts. Poly(A)-RNA from clones Lov1, 4, and 8 and control clone M25-3 were electrophoresed on a 1.5% agarose gel containing 10 mM methylmercury hydroxide (23), transferred to diazobenzyloxymethyl-paper (24), and hybridized with a nick-translated probe derived from pCR1ov2.1 (Hhaov, see ref. 10). Lane 1: positions of size markers are indicated by arrowheads (from top to bottom): 28S, 23S, 18S, and 16S ribosomal RNAs from mouse or *Escherichia coli*. Lanes 2–4: poly(A)-RNA from clones Lov8, Lov1, and Lov4, respectively. Lane 5: purified ov-mRNA mixed with RNA from the control clone M25-3. (*C*) S1 nuclease mapping of 5′ terminus of ovalbumin transcripts. Poly(A)-RNA from clones Lov1 and Lov8 was annealed to an *Ava* II–*Pst*4 fragment of pBov1 (see Fig. 1*a*), 5′-end-labeled at the *Ava* II site only, before S1 digestion and electrophoresis on an 8% acrylamide/7 M urea gel. Lanes 1 and 2: ov-mRNA hybridized to pBov2 (5′-end-labeled at the *Xba* I site), and then treated as described in the legend to Fig. 5*A* was used to provide a marker size and a control demonstrating that the S1 digestion was efficient. Lanes 3–5: RNA from clones Lov1 and 8 and control clone M25-3, respectively. The position of size markers from a *Hin*dIII digest of simian virus 40 DNA is indicated by arrowheads. Size of bands (in kb): 1.77, 1.17, 0.52, 0.45, and 0.21 from top to bottom, respectively.

DISCUSSION

The cloned ovalbumin gene has been introduced into mouse L cells and maintained therein for 6 months without loss or rearrangement. The ovalbumin gene copies could be integrated into host cell sequences, as previously suggested (25), or they could be associated with the carrier DNA used in the transformation to form some sort of "double minute-like" chromosomes (26). At least some of the ovalbumin gene copies are transcribed. We estimate that clone Lov8 contains about five copies of transcript per cell and that this copy number is lower in other clones. In general, there is little correlation between the gene and transcript copy numbers, as is also the case for the rabbit β-globin gene introduced in the same way into L cells (13).

The L cell ovalbumin transcript appears to be mostly polyadenylylated and to contain correctly joined transcripts of ovalbumin gene exons 1–7; it can protect a full-length ds cDNA from nuclease digestion. We have not determined whether this poly(A)-RNA is associated with polysomes and is translated. The 3′ terminus of the L cell transcripts corresponds (within the sensitivity of the technique used) to that of ov-mRNA, but the transcript, which is transcribed from the same strand as ov-

mRNA, is 650 ± 150 nucleotides longer than ov-mRNA. These additional nucleotides should therefore be located at the 5′ end of the transcripts, a conclusion that is supported by the finding that there are approximately equimolar transcripts up to the 3′ end of the gene and extending 550 nucleotides beyond the *Ava* II site (Fig. 1*a*), which is itself 130 bp upstream from the ovalbumin leader-coding sequences. A probable map of the L cell transcript is shown in Fig. 1*b*. The region of the transcript corresponding to the sequences between the 3′ end of the leader (L) and the *Ava* II site has not been blocked in, because we are not sure whether this region is present in the 2500-nucleotide-long "ovalbumin" RNA present in L cells. (If it contained an appropriate splice point, the transcript of sequences upstream from the *Ava* II site could be spliced in this region directly to the exon 1 transcript.) In any case, the L cell ovalbumin gene transcript has probably been processed from a nuclear precursor by polyadenylylation and splicing, as is the case for ov-mRNA in chicken oviduct (27, 28). Our results show that an avian transcript can be correctly spliced in a mouse cell. Our results also show that the avian polyadenylylation signal very likely is correctly recognized, although additional studies are required to definitely establish this point. Recent results from other laboratories have shown that rabbit splicing and polyadenylylation signals are recognized in mouse cells (7, 13), and mouse signals in monkey cells (29). There thus appears to be little cell-type and species variation in polyadenylylation and splicing signals within the animal kingdom, which fits with our previous report that all exon–intron junctions are related in sequence (11).

On the other hand, it appears from the noncoincidence of the 5′ termini of the L cell transcripts and ov-mRNA that the chicken promoter is not recognized by the mouse RNA polymerase. However, although we did not find any evidence for transcription of the *Ava* II–*Pst*4 (Fig. 1*a*) region in chicken oviduct nuclei *in vivo* or *in vitro* (ref. 27, and M. LeMeur, R. Palmiter, and P. Chambon, unpublished results), it has not been unequivocally demonstrated that initiation of transcription of the ovalbumin gene in the oviduct occurs at the base pair encoding the first nucleotide of ov-mRNA. Thus, the L cell transcript and ov-mRNA may be read from a common promoter, but have different processing at their 5′ ends. Alternatively, the promoter used in L cells could be an artifactual one, corresponding, for instance, to a particularly A·T-rich region. (In fact, our results do not exclude that 5% or less of the transcripts could have 5′ termini coinciding with that of ov-mRNA.) Functioning of the ovalbumin gene promoter in oviduct cells is dependent on hormonal stimulation. The appropriate receptors or other hormone-related factors might be required in order for RNA polymerase to recognize the correct initiation site.

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1. Gannon, F., O'Hare, K., Perrin, F., LePennec, J. P., Benoist, C., Cochet, M., Breathnach, R., Royal, A., Garapin, A., Cami, B. & Chambon, P. (1979) *Nature (London)* **278**, 428–434.
2. Palmiter, R. D. (1975) *Cell* **4**, 189–197.
3. Birnstiel, M. & Chipchase, M. (1977) *Trends Biochem. Sci.* **2**, 149–152.
4. Weissmann, C. (1978) *Trends Biochem. Sci.* **3**, N109–N111.
5. Wigler, M., Pellicer, A., Silverstein, S. & Axel, R. (1978) *Cell* **14**, 725–731.
6. Wigler, M., Sweet, T., Sim, G. K., Wold, B., Pellicer, A., Lacy, E., Maniatis, T., Silverstein, S. & Axel, R. (1979) *Cell* **16**, 777–785.
7. Weissmann, C., Mantei, N., Boll, W., Taniguchi, R., Van Ooyen, R., van der Berg, J., Fried, M. & Murry, K. (1979) in *From Gene to Protein: Transfer in Normal and Abnormal Cells*, Proceedings of the 11th Miami Winter Symposium, ed. Russell, T. R. (Academic, New York), pp. 99–132.
8. Boll, W., Nantei, N., Wilkie, N., Clements, B., Greenaway, P. & Weissmann, C. (1979) *Experientia* **35**, 957–962.
9. Humphries, P., Cochet, M., Krust, A., Gerlinger, P., Kourilsky, P. & Chambon, P. (1977) *Nucleic Acids Res.* **4**, 2389–2405.
10. Breathnach, R., Mandel, J. L. & Chambon, P. (1977) *Nature (London)* **270**, 314–319.
11. Breathnach, R., Benoist, C., O'Hare, K., Gannon, F. & Chambon, P. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4853–4857.
12. Mandel, J. L., Breathnach, R., Gerlinger, P., LeMeur, M., Gannon, F. & Chambon, P. (1978) *Cell* **14**, 641–653.
13. Mantei, N., Boll, W. & Weissmann, C. (1979) *Nature (London)* **281**, 40–46.
14. Scherrer, K. & Darnell, J. D. (1962) *Biochem. Biophys. Res. Commun.* **7**, 486–490.
15. Berk, A. J. & Sharp, P. A. (1977) *Cell* **12**, 721–732.
16. Sanger, F. & Coulson, A. R. (1977) *FEBS Lett.* **87**, 107–110.
17. O'Hare, K., Breathnach, R., Benoist, C. & Chambon, P. (1979) *Nucleic Acids Res.* **7**, 321–324.
18. Graham, F. L. & Van der Eb, A. J. (1973) *Virology* **52**, 456–467.
19. Southern, E. M. (1975) *J. Molec. Biol.* **98**, 503–518.
20. Weaver, R. & Weissmann, C. (1979) *Nucleic Acids Res.* **7**, 1175–1193.
21. Casey, J. & Davidson, N. (1977) *Nucleic Acids Res.* **4**, 1539–1552.
22. Benoist, C., O'Hare, K., Breathnach, R. & Chambon, P. (1980) *Nucleic Acids Res.*, in press.
23. Bailey, J. M. & Davidson, N. (1976) *Anal. Biochem.* **70**, 75–85.
24. Alwine, J. C., Kemp, D. J. & Stark, G. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5350–5354.
25. Pellicer, A., Wigler, M., Axel, R. & Silverstein, S. (1978) *Cell* **14**, 133–141.
26. Balaban-Melenbaum, G. & Gilbert, F. (1977) *Science* **198**, 739–741.
27. Chambon, P., Benoist, C., Breathnach, R., Cochet, M., Gannon, F., Gerlinger, P., Krust, A., LeMeur, M., LePennec, J. P., Mandel, J. L. O'Hare, K. & Perrin, F. (1979) in *From Gene to Protein: Transfer in Normal and Abnormal Cells*, Proceedings of the 11th Miami Winter Symposium, ed. Russell, T. R. (Academic, New York), pp. 55–82.
28. Roop, D. R., Nordstrom, J. L., Tsai, S. Y., Tsai, M. J. & O'Malley, B. W. (1978) *Cell* **15**, 671–685.
29. Hamer, D. H. & Leder, P. (1979) *Nature (London)* **281**, 35–40.