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Biochemistry

Chicken lysozyme gene contains several intervening sequences

(gene organization/recombinant plasmid/Southern hybridization/cell differentiation)

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ABSTRACT **The organization of the chicken lysozyme gene and its neighboring sequences was examined by a comparison of the restriction map of the lysozyme structural gene with the map of the lysozyme gene in genomic DNA. Chicken DNA was cleaved with restriction endonucleases and the DNA fragments were separated by agarose gel electrophoresis. After transfer of the fragments onto nitrocellulose filters, those fragments that contain lysozyme mRNA sequences were detected by hybridization of the filters to labeled probes generated from pls-1, a recombinant plasmid carrying the lysozyme structural gene. This analysis revealed the presence of at least three intervening sequences, two of which interrupt the protein coding region and one of which is located in the 3′ untranslated region. When oviduct DNA and sperm DNA were compared, no difference was observed in the size and number of restriction fragments that contain either lysozyme or ovalbumin structural gene sequences.**

In the chicken oviduct the synthesis of the egg white proteins ovalbumin, conalbumin, ovomucoid, and lysozyme is coordinatedly controlled by steroid hormones (1). By using DNA complementary to highly purified mRNAs, it has been shown that the steroid-controlled rate of protein synthesis is closely correlated with the cellular concentrations of the ovalbumin mRNA (2–6), the conalbumin mRNA (7, 8), and the ovomucoid and lysozyme mRNA (6, 9). Using isolated nuclei to determine the rates of synthesis of the egg white protein mRNA sequences (10), we have shown that the increased level of these mRNAs in the target cell after steroid induction results from transcriptional activation of the egg white protein genes (8, 9, 11). We suspect that the coordinated control of expression of the egg white protein genes is based, at least in part, on the sequence and organization of the DNA within and around the genes coding for these proteins. A comparison between the structure of the ovalbumin gene, which is known already in some detail (12–18), with that of the other egg white protein genes will most likely reveal information about how steroid hormones control gene expression in a coordinated fashion. To provide pure molecular probes for the analysis of the organization of the egg white protein genes in genomic DNA, we have prepared recombinant plasmids containing DNA complementary to the egg white protein mRNAs. Among others, a clone containing extensive portions of the lysozyme structural gene has been obtained (ref. 19 and unpublished results). The cloned lysozyme DNA was used to examine the organization of the lysozyme gene in genomic DNA by using the Southern technique (20). We report here that the linear organization of lysozyme structural gene sequences is not continuous in chicken oviduct and sperm DNA but is interrupted by at least three intervening sequences.

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MATERIALS AND METHODS

Hybridization Probes. DNA from pls-1, a recombinant plasmid containing lysozyme mRNA sequences (19), and DNA from pOV230, a recombinant plasmid containing ovalbumin mRNA sequences (21), were labeled to high specific activities ($6–10 \times 10^7$ cpm/μg) by nick-translation (22). The reaction conditions were as described by Weinstock *et al.* (14), except that [α-^{32}P]dCTP [Amersham Buchler, 350 Ci/mmol (1 Ci = 3.7×10^{10} becquerels)] was used as the radioactive label. The plasmid pls-1 was constructed in our laboratory. pOV230 DNA digested with restriction endonuclease *Hae* III was kindly provided by B. O'Malley. To prepare specific probes for the 5′ and 3′ part of the lysozyme mRNA sequence, pls-1 DNA was cleaved with *Hin*fI. The two fragments that contain lysozyme mRNA sequences [440 base pairs (bp) and 1720 bp; Fig. 1] were separated by electrophoresis on a 5% polyacrylamide slab gel, recovered from the excised gel sections by diffusion as described (19), and labeled by nick-translation.

Restriction Cleavage of Chicken DNA and Southern Hybridization. HNL laying hens and cockerels were used in all studies. Oviduct nuclei were prepared as previously described (10). After the Triton wash, the nuclear pellet was resuspended in 10 mM Tris·HCl, pH 7.5/100 mM NaCl/5 mM EDTA (TNE) and high molecular weight DNA was extracted as described by Gross-Bellard *et al.* (23). Sperm was collected from cockerels, washed in TNE containing 30% (vol/vol) glycerol, and pelleted by centrifugation at $3000 \times g$. The pellet was resuspended in TNE with a Teflon/glass homogenizer and the DNA was extracted as above. Restriction cleavage of chicken DNA and subsequent electrophoresis on 0.8–2% agarose gels were performed as described (19). After denaturation *in situ* and transfer of the DNA onto nitrocellulose filters as described by Southern (20), the filters were pretreated, the DNA on them was hybridized, and the filters were washed and autoradiographed as described by Jeffreys and Flavell (24). Hybridization was carried out for 18–20 hr with ^{32}P-labeled pls-1 DNA or pOV230 DNA (20 ng/ml) or with the labeled 5′ and 3′ specific lysozyme probes (8 ng/ml).

RESULTS

Localization of lysozyme structural gene sequences in genomic DNA

To analyze the organization of the lysozyme gene and its surrounding sequences, we have compared the restriction map of the lysozyme structural gene with the map of the lysozyme gene in genomic DNA. The map of the lysozyme structural gene (Fig. 1) was determined from an *in vitro* synthesized double-stranded DNA copy of lysozyme mRNA and from pls-1, a recombinant plasmid constructed in our laboratory and con-

Abbreviations: kb, kilobase (1000 base pairs); bp, base pairs; TNE, Tris/NaCl/EDTA.

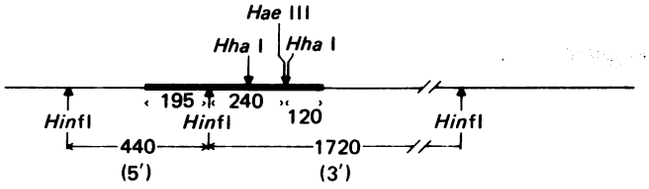

FIG. 1. Restriction map of lysozyme structural gene sequences contained in the recombinant plasmid pls-1 (19). The thin line represents pBR322 DNA; the thick line represents lysozyme specific sequences in pls-1. The cloned DNA contains 555 bp coding for 136 amino acids of prelysozyme and for the entire 3′ noncoding part of the mRNA. It lacks the sequences corresponding to the 5′ untranslated region of the mRNA and to the first 11 amino acids of prelysozyme. *Eco*RI, *Bam*HI, *Hin*dIII, and *Pst* I do not cut the lysozyme structural gene contained in pls-1. The sites of cleavage of pls-1 DNA by *Hin*fI, *Hae* III, and *Hha* I are indicated by arrows. The numbers between the arrows indicate the length of the fragments in base pairs. The 440-bp and 1720-bp *Hin*fI fragments have been used as hybridization probes specific for the 5′ and 3′ parts of the lysozyme mRNA sequence.

taining extensive parts of lysozyme structural gene sequences (19). The organization of the lysozyme gene in genomic DNA was analyzed by the Southern technique (20) with ^{32}P-labeled pls-1 DNA as a hybridization probe. If the linear organization of lysozyme structural gene sequences in genomic DNA is identical to that in the mature lysozyme mRNA and if there is one gene copy per haploid genome, the cleavage of genomic DNA with enzymes that do not cut the lysozyme structural gene—i.e., *Eco*RI, *Hin*dIII, *Bam*HI, and *Pst* I—should generate a single DNA fragment containing the entire structural gene. However, cleavage with *Eco*RI yielded a 6.3-kilobase (kb) and a 2.3-kb fragment that annealed to pls-1 (Fig. 2*A*, slot 1); cleavage with *Hin*dIII yielded a 3.0-kb, a 2.0-kb, and a 0.8-kb fragment that annealed to pls-1 (Fig. 2*A*, slot 3), and cleavage with *Pst* I yielded a 3.8-kb, a 1.5-kb, and a 0.8-kb fragment that annealed to pls-1 (Fig. 2*B*, slot 1). Cleavage of genomic DNA with *Bam*HI resulted in a single fragment 9.8 kb in length that hybridized to pls-1 (Fig. 2*A*, slot 2). Cleavage of genomic DNA

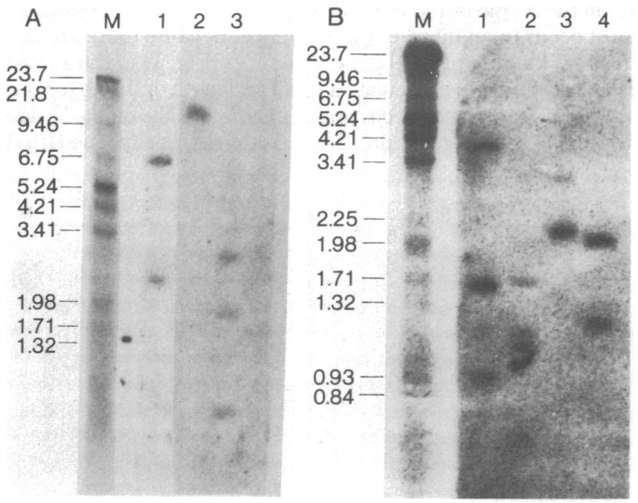

FIG. 2. Detection of DNA fragments containing lysozyme structural gene sequences generated by restriction cleavage of chicken oviduct DNA. DNA was cleaved, electrophoresed, transferred onto nitrocellulose filters, and hybridizided to ^{32}P-labeled pls-1 DNA. Cleavage was performed with *Eco*RI (*A*, slot 1), *Bam*HI (*A*, slot 2), *Hin*dIII (*A*, slot 3), *Pst* I (*B*, slot 1), *Hin*fI (*B*, slot 2), *Hha* I(*B*, slot 3) and *Hae* III (*B*, slot 4). Nick-translated λ [^{32}P]DNA cleaved with *Hin*dIII and *Eco*RI plus *Hin*dIII was used as molecular weight marker (slots M). The lengths of the markers are given in kb as measured by Murray and Murray (25).

with *Hin*fI and *Hae* III (enzymes that cut the lysozyme structural gene once, generating a predicted yield of two lysozyme genomic DNA fragments) resulted in four lysozyme DNA fragments 1.6 kb, 1.2 kb, 1.1 kb, and 0.5 kb in length and three lysozyme DNA fragments 2.1 kb, 1.2 kb, and 0.4 kb in length, respectively (Fig. 2*B*, slots 2 and 4, and Fig. 3*B*, slot 4).

The difference between the observed annealing pattern and that predicted from the map of the structural gene could be explained in different ways: (*i*) the lysozyme gene may be present in more than one copy per haploid genome; (*ii*) the sequences flanking the lysozyme gene may differ in alleles of the gene or in different oviduct cell types; (*iii*) other sequences in chicken DNA may have some partial homology with pls-1; (*iv*) the lysozyme gene may consist of segments derived from various parts of the chicken genome; and (*v*) the lysozyme gene may consist of structural gene sequences alternating with intervening sequences.

Intervening sequences within the lysozyme gene

We have shown that the lysozyme structural gene sequences are interrupted by intervening sequences in genomic DNA by using hybridization probes specific for the 5′ and 3′ parts of the lysozyme mRNA sequence. These probes have been prepared by cleavage of pls-1 with *Hin*fI (Fig. 1). If lysozyme structural gene sequences in genomic DNA are interrupted, each lysozyme DNA fragment observed should contain only a portion of the lysozyme mRNA sequence. In this case, if a genomic DNA fragment contains the *Hin*fI site corresponding to that of the structural gene (in the following referred to as intragenic endonuclease site), it should anneal to both probes. Otherwise it should anneal to either the 5′ or the 3′ probe. The hybridization patterns obtained with the 5′ and 3′ probes are shown in Fig. 3. The 6.3-kb *Eco*RI fragment hybridized to both probes, while the 2.3-kb fragment hybridized only to the 3′ probe (Fig. 3*A*, slots 1). The 2.0-kb *Hin*dIII fragment hybridized to the 5′ probe, the 3.0-kb *Hin*dIII fragment hybridized to the 3′ probe, and the 0.8-kb fragment hybridized to both probes (Fig. 3*A*, slots 3). Similarly, the 0.8-kb *Pst* I fragment hybridized to the 5′ probe, the 3.8-kb *Pst* I fragment hybridized to the 3′ probe, and the 1.5-kb *Pst* I fragment hybridized to both probes (Fig. 3*B*, slots 1). These results indicate the presence of inserts containing *Hin*dIII and *Pst* I sites on the 5′ side and inserts containing *Eco*RI, *Hin*dIII, and *Pst* I sites on the 3′ side of the intragenic *Hin*fI site. The 9.8-kb *Bam*HI fragment hybridized to both the 5′ and 3′ probe (Fig. 3*A*, slots 2), and digestion with *Bam*HI plus *Eco*RI or *Hin*dIII (Fig. 4*B*) confirms that all lysozyme structural gene sequences are contained within this single 9.8-kb fragment.

Unambiguous evidence for the presence of at least three intervening sequences was obtained by cleavage with *Hin*fI, *Hae* III, and *Hin*fI plus *Hae* III. *Hin*fI cleavage of genomic DNA yielded four lysozyme DNA fragments (Fig. 3*B*, slots 2). The 1.1-kb and 0.5-kb fragments hybridized to the 5′ probe, and the 1.6-kb and 1.2-kb fragments hybridized to the 3′ probe. None of the *Hin*fI fragments hybridized to both probes. These data indicate the presence of two intervening sequences, one on the 5′ side and one on the 3′ side of the intragenic *Hin*fI site. Cleavage with *Hae* III resulted in three lysozyme DNA fragments (Fig. 3*B*, slots 4). The 1.2-kb fragment annealed to the 5′ probe, the 0.4-kb fragment annealed to the 3′ probe, and the 2.1-kb fragment annealed to both probes. Therefore, the 5′ to 3′ order of the *Hae* III fragments must be 1.2-kb, 2.1-kb, and 0.4-kb. In the lysozyme mRNA sequence, the unique *Hae* III site is on the 3′ side of the unique *Hin*fI site (Fig. 1). This intragenic *Hae* III site should correspond to the junction of the 2.1-kb and the 0.4-kb lysozyme DNA fragments generated by *Hae* III cleavage of genomic DNA. An intervening sequence

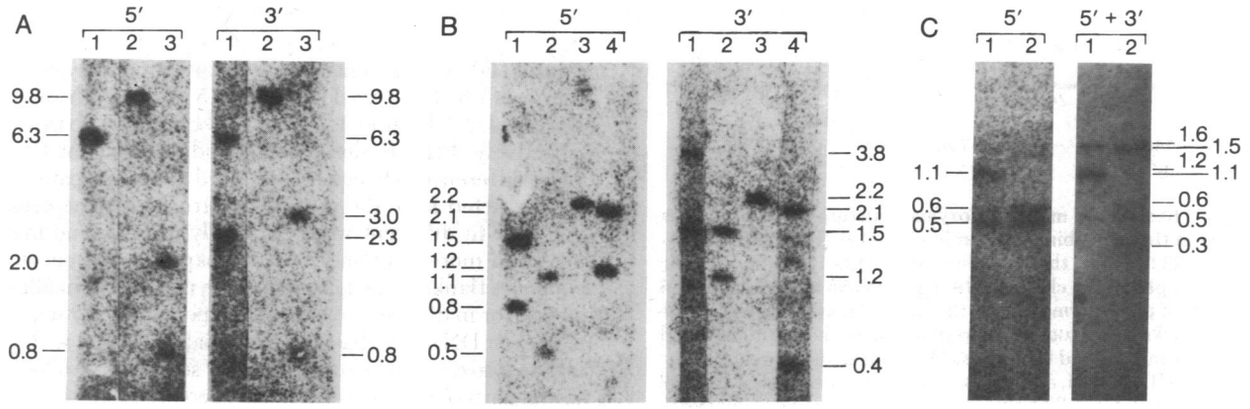

FIG. 3. Detection of DNA fragments containing the 5′ and 3′ parts of lysozyme structural gene sequences generated by restriction cleavage of oviduct DNA. DNA was cleaved, electrophoresed, transferred onto nitrocellulose filters, and hybridized to the ^{32}P-labeled probes specific for the 5′ and 3′ part of the lysozyme mRNA sequence as indicated. These probes were prepared from pls-1 DNA cleaved with *Hin*fI (Fig. 1). Oviduct DNA was cleaved with *Eco*RI (*A*, slots 1), *Bam*HI (*A*, slots 2), *Hin*dIII (*A*, slots 3), *Pst* I (*B*, slots 1), *Hin*fI (*B*, slots 2), *Hha* I (*B*, slots 3), *Hae* III (*B*, slots 4), *Hin*fI (*C*, slots 1) and *Hin*fI/*Hae* III (*C*, slots 2). The sizes of the fragments were determined as described in the legend to Fig. 2 and are given in kb. In order to detect the 0.4-kb *Hae* III fragment (*B*, slot 4), the last washing step of filters in 0.015 M NaCl/0.0015 M sodium citrate had to be omitted. Otherwise this 0.4-kb band appeared very faint (Fig. 4*A*, slot 9) or was not detectable (Fig. 2*B*, slot 4).

containing a further *Hae* III site must be present on the 5′ side of the intragenic *Hin*fI site. Digestion with *Hin*fI plus *Hae* III yielded four lysozyme DNA fragments (Fig. 3*C*, slots 2). The 0.6-kb and the 0.5-kb fragments hybridized to the 5′ probe and the 1.5-kb and the 0.3-kb fragments hybridized to the 3′ probe (Fig. 5, slots 4). No 0.24-kb fragment was found, as would be predicted from the map of the lysozyme mRNA sequence (Fig. 1). The 1.5-kb *Hin*fI/*Hae* III fragment must be derived from the 2.1-kb *Hae* III fragment, and, because it hybridized only to the 3′ probe, its *Hae* III site should correspond to the intragenic *Hae* III site. Because the 2.1-kb *Hae* III fragment hybridized to both the 5′ and 3′ probe and therefore contains the intragenic *Hin*fI site, the 1.5-kb *Hin*fI/*Hae* III fragment is derived from the 2.1-kb *Hae* III fragment by cleavage, most likely, at the intragenic *Hin*fI site. Indeed, a 0.6-kb *Hin*fI/*Hae* III fragment was also observed. Hence, the 1.5-kb *Hin*fI/*Hae* III fragment should be derived by cleavage at the unique intragenic *Hin*fI and *Hae* III site. Comparing the size of the 1.5-kb *Hin*fI/*Hae* III lysozyme fragment of genomic DNA (Fig. 6) with the size of the corresponding 0.24-kb *Hin*fI/*Hae* III fragment of the lysozyme mRNA sequences (Fig. 1), we calculate that 1.26 kb of intervening sequences occur in genomic DNA between the intragenic *Hin*fI and *Hae* III sites.

The 1.5-kb *Hin*fI/*Hae* III lysozyme fragment must be derived from cleavage of the 1.6-kb *Hin*fI fragment by *Hae* III. This locates a *Hin*fI site 1.6 kb on the 3′ side of the intragenic *Hin*fI site. This *Hin*fI site is located in an intervening sequence on the 3′ side of the intragenic *Hae* III site and is likely to be at the junction of the 1.6-kb and 1.2-kb *Hin*fI fragments. The 0.3-kb *Hin*fI/*Hae* III fragment could then be derived from *Hin*fI cleavage of the 0.4-kb *Hae* III fragment and from *Hae* III cleavage of the 1.2-kb *Hin*fI fragment (Fig. 6). This 0.4-kb *Hae* III fragment most likely contains all the 100 bp of the structural gene sequences located between the intragenic *Hae* III site and the 3′ end of the mRNA sequence (Fig. 1). Therefore, we calculate a maximal length of 0.3 kb for this intervening sequence occurring in genomic DNA on the 3′ side of the intragenic *Hae* III site.

The 0.6-kb *Hae* III/*Hin*fI fragment hybridized to the 5′ probe and should be derived from *Hin*fI cleavage of the 2.1-kb *Hae* III fragment and by *Hae* III cleavage of the 1.1 kb *Hin*fI fragment. This locates a *Hin*fI site 1.1 kb and a *Hae* III site 0.6 kb on the 5′ side of the intragenic *Hin*fI site (Fig. 6). These sites must occur in an intervening sequence. Because there are less than 0.2 kb of structural gene sequences (Fig. 1) in the 1.1-kb *Hin*fI fragment, we calculate a minimal length of 0.9 kb for this intervening sequence occurring in genomic DNA on the 5′ side of the intragenic *Hin*fI site. The 0.5-kb fragment of the *Hin*fI

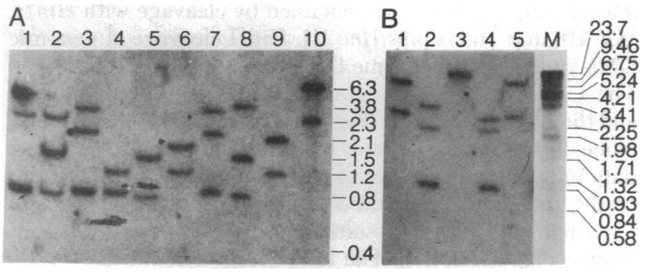

FIG. 4. Hybridization of oviduct DNA digested with various combinations of endonucleases to ^{32}P-labeled pls-1 DNA. (*A*) Cleavage was performed with *Pst* I/*Hin*dIII (slot 1), *Eco*RI/*Pst* I (slot 2), *Bam*HI/*Hin*dIII (slot 3), *Hae* III/*Hin*dIII (slot 4), *Hae* III/*Pst* I (slot 5), *Eco*RI/*Hae* III (slot 6), *Hin*dIII (slot 7), *Pst* I (slot 8), *Hae* III (slot 9), and *Eco*RI (slot 10). (*B*) Cleavage was performed with *Eco*RI (slot 1), *Hin*dIII (slot 2), *Bam*HI (slot 3), *Eco*RI/*Hin*dIII (slot 4), and *Bam*HI/*Eco*RI (slot 5). The molecular weight markers (slot M) used were those described in Fig. 2.

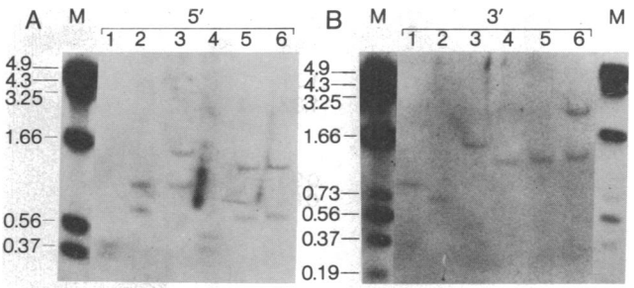

FIG. 5. Hybridization of oviduct DNA digested with various combinations of endonucleases to the 5′ (*A*) and 3′ (*B*) probes. Cleavage was performed with *Hin*dIII/*Hin*fI (slots 1), *Hae* III/*Hin*dIII (slots 2), *Eco*RI/*Hae* III (slots 3), *Hin*fI/*Hae* III (slots 4), *Hae* III/*Pst* I (slots 5), and *Eco*RI/*Pst* I (slots 6). As molecular weight markers (M) 0.2 ng of a mixture of different restriction digests of pls-1 DNA was used. These markers were readily detected after hybridization to the labeled probes. Their lengths (19) are given in kb. It should be noted that the 0.73-kb and the 0.19-kb fragments hybridized only the 3′ probe because they contain only the 3′ part of the lysozyme mRNA sequence.

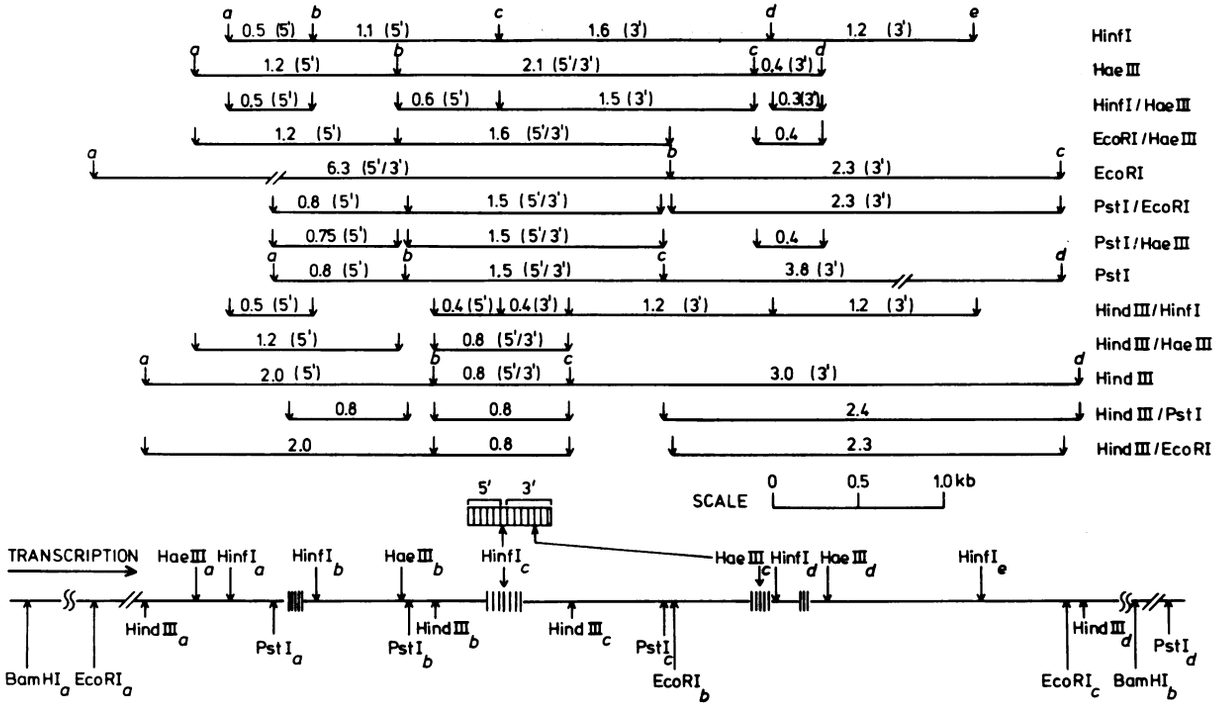

FIG. 6. Preliminary map of the chicken lysozyme gene. The sizes and relative locations of the lysozyme specific DNA fragments observed in Figs. 3, 4, and 5 are summarized in the upper part and have been used to construct the map of the lysozyme gene shown in the lower part. Endonuclease sites on the DNA are shown by arrows and indexed by a, b, c . . . according to their relative 5′ to 3′ order. With this nomenclature the genomic *Hin*fI$_c$ and *Hae* III$_c$ sites would correspond to the *Hin*fI and *Hae* III sites of the lysozyme structural gene. The numbers represent the lengths of the DNA fragments in kb. (5′), (3′), and (5′/3′) indicate the hybridization of a fragment to the 5′, 3′, or both the 5′ and 3′ probe, respectively. Lysozyme structural gene sequences are represented by ||||. Intervening sequences and flanking DNA sequences are represented by —. It should be noted that our analysis is restricted to lysozyme mRNA sequences contained in the recombinant plasmid pls-1 and that the four blocks of lysozyme structural gene sequences shown in this figure might be further interrupted by intervening sequences.

plus *Hae* III digest is identical to the 0.5-kb *Hin*fI fragment and should be derived from cleavage at two *Hin*fI sites within the 1.2-kb *Hae* III fragment.

Preliminary restriction map of the lysozyme gene

From the data presented in the previous section, a map of *Hin*fI and *Hae* III cleavage sites within and around the lysozyme gene has been deduced (Fig. 6). Relative to these sites the *Bam*HI, *Eco*RI, *Hin*dIII, and *Pst* I sites were mapped by digestion of chicken DNA with various combinations of endonucleases and subsequent hybridization of the fragments to pls-1 (Fig. 4) and to the 5′ and 3′ probes (Fig. 5).

All the data have been used to construct a preliminary map of the lysozyme gene (Fig. 6). The 2.1-kb *Hae* III$_{b-c}$ fragment was cleaved by *Eco*RI to produce a 1.6-kb fragment (Fig. 4*A*, slots 6, 9, 10) that hybridized to both the 5′ and 3′ probe (Fig. 5, slots 3). Therefore, an *Eco*RI site should map at 1.6 kb on the 3′ side of the *Hae* III$_b$ site (Fig. 6). Digestion of genomic DNA with *Pst* I plus *Hae* III (Fig. 4*A*, slots 5, 8, 9, and Fig. 5, slots 5) shows that the 0.8-kb *Pst* I fragment is almost entirely contained within the 1.2-kb *Hae* III$_{a-b}$ fragment and that the 1.5-kb *Pst* I fragment is located within the 2.1-kb *Hae* III$_{b-c}$ fragment. Furthermore, the 2.3-kb *Eco*RI$_{b-c}$ fragment is contained within the 3.8-kb *Pst* I fragment (Fig. 4*A*, slots 2, 8, 10, and Fig. 5, slots 6). Because the *Eco*RI$_b$ site maps 1.6 kb on the 3′ side of *Hae* III$_b$, the *Pst* I$_c$ site should map between 1.5 kb and 1.6 kb on the 3′ side of *Hae* III$_b$ (Fig. 6). Cleavage of genomic DNA with *Hin*dIII plus *Hae* III shows that the 0.8-kb *Hin*dIII fragment is contained within the 2.1-kb *Hae* III$_{b-c}$ fragment and that the 1.2-kb *Hae* III$_{a-b}$ fragment is contained within the 2.0-kb *Hin*dIII fragment (Fig. 4*A*, slots 4, 7, 9, and Fig. 5, slots 2). The 0.8-kb *Hin*dIII fragment is cleaved by *Hin*fI to generate two 0.4-kb fragments hybridizing to either the 5′ or the 3′ probe (Fig. 5, slots 1). Therefore, the *Hin*dIII sites in intervening DNA should map 0.4 kb on the 5′ side and on the 3′ side of *Hin*fI$_c$ (Fig. 6).

Fragments generated from two restriction cuts within one intervening sequence would not be detected by hybridization to structural gene sequences. However, because the *Hin*fI$_{b-c}$ and the *Hin*fI$_{c-d}$ fragments are contiguous as well as the *Hae* III$_{b-c}$ and the *Hae* III$_{c-d}$ fragments, the *Hin*fI$_b$–*Hae* III$_d$ segment should be 3.0 kb in length. We have mapped the restriction sites *Pst* I$_b$, *Pst* I$_c$, *Hin*dIII$_b$, *Hin*dIII$_c$, and *Eco*RI$_b$ within this 3.0-kb segment. The location of the *Pst* I$_d$, *Hin*dIII$_d$, and *Eco*RI$_c$ sites in the 3′ flanking sequences can then be determined (Fig. 6). The simplest way to map the *Hin*fI, *Hae* III, *Pst* I, and *Hin*dIII sites in the 5′ flanking sequences is to assume that they are nearest to the 3.0-kb *Hin*fI$_b$–*Hae* III$_d$ segment, as shown in Fig. 6. However, we cannot rigorously exclude a discontinuity of the map at the 5′ side of the *Hin*fI$_b$ site. The map of the chicken lysozyme gene shown in Fig. 6 is the simplest one consistent with all our data.

Organization of the lysozyme and ovalbumin genes in sperm DNA

The analysis of the lysozyme gene described above was carried out with hen oviduct DNA. Using the same technique, we have also studied the organization of the lysozyme gene and, for comparison, the ovalbumin gene in sperm DNA. The hybridization patterns are shown in Fig. 7. No difference in the number and size of lysozyme specific fragments from sperm and oviduct DNA has been found. Also all the ovalbumin specific fragments from oviduct DNA (refs. 12–15 and unpublished results) are present in sperm DNA. In a recent report (14), it had been shown that cleavage of oviduct and sperm DNA with *Eco*RI and *Hin*dIII generated identical ovalbumin specific fragments.

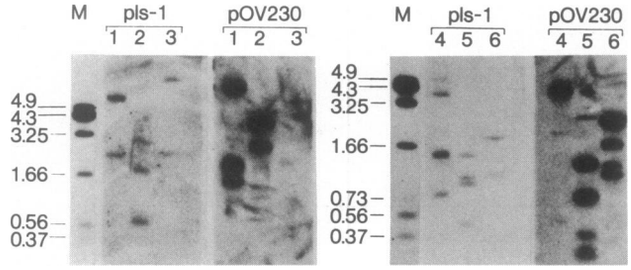

FIG. 7. Detection of DNA fragments containing lysozyme and ovalbumin structural gene sequences generated by restriction cleavage of chicken sperm DNA. Sperm DNA was cleaved, electrophoresed, transferred onto nitrocellulose filters, and hybridized to ^{32}P-labeled pls-1 or pOV230 DNA as indicated. *Eco*RI digestion (slots 1) generated two lysozyme DNA fragments (6.6 kb and 2.4 kb) and three ovalbumin DNA fragments (9.0 kb, 2.3 kb, and 1.7 kb). *Hin*dIII digestion (slots 2) generated three lysozyme DNA fragments (3.0 kb, 2.0 kb, and 0.8 kb) and two ovalbumin DNA fragments (4.9 kb and 3.2 kb). *Bam*HI digestion (slots 3) generated one lysozyme DNA fragment (10 kb). *Pst* I digestion (slots 4) generated three lysozyme DNA fragments (3.9 kb, 1.5 kb, and 0.8 kb) and one ovalbumin DNA fragment (4.4 kb). *Hin*fI digestion (slots 5) generated four lysozyme DNA fragments (1.5 kb, 1.05 kb, 0.95 kb, and 0.5 kb) and six ovalbumin DNA fragments (2.8 kb, 1.4 kb, 0.8 kb, 0.4 kb, 0.3 kb, and 0.2 kb). *Hae* III digestion (slots 6) generated two lysozyme DNA fragments (2.0 kb and 1.1 kb) and four ovalbumin DNA fragments (2.8 kb, 1.9 kb, 1.3 kb, and 1.1 kb). The molecular weight markers (slots M) used were those described in Fig. 5.

DISCUSSION

The presented data show that the lysozyme structural gene sequences in chicken DNA are interrupted by at least three intervening sequences. One intervening sequence is on the 5′ side of the intragenic *Hin*fI site, another is between the intragenic *Hin*fI and *Hae* III sites. A third one is on the 3′ side of the intragenic *Hae* III site and therefore is located within the 3′ untranslated part of the lysozyme mRNA (19). The lengths of the three intervening sequences are $\geq$0.9 kb, 1.25 kb, and $<$0.3 kb, respectively. The presence of intervening sequences has been observed in several genes of adenovirus and simian virus 40, in yeast tRNA genes, in the 28S rRNA gene of *Drosophila melanogaster*, and in the genes coding for rabbit and mouse β-globin, mouse immunoglobulin light chain, and chicken ovalbumin (for references, see ref. 17). The biological function of intervening sequences is still unknown. While the lysozyme mRNA is about 0.6 kb in length (26), the lysozyme gene in chicken DNA has a minimal length of 3.3 kb (from *Pst* I_a to *Hae* III_d; Fig. 6). The existence of a large precursor of lysozyme mRNA is a possibility worth investigating. Our results indicate that the organizations of the lysozyme gene and the ovalbumin gene are identical in the male gametes and in the oviduct cells.

In the tubular gland cells of the oviduct, the transcription of the genes coding for the egg white proteins is coordinately controlled by steroid hormones (8, 9, 11). One way to achieve this control would be to group these genes into a structural and functional unit. A close physical linkage of the egg white protein genes is unlikely, because all the restriction fragments we observed thus far (Fig. 7 and unpublished results) hybridized exclusively with either ovalbumin, ovomucoid, or lysozyme specific DNA. Another possibility would be to provide each gene with the ability to recognize the same regulatory molecules. The molecular cloning and the comparative structural and functional analysis of the egg white protein genes will help us understand how coordinated gene expression is controlled.

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