Volume 9 Number 15 1981 Nucleic Acids Research

Isolation and characterization of the complete chicken β-globin gene region: frequent deletion of the adult β-globin genes in λ

Bryant Villeponteau and Harold Martinson

Division of Biochemistry, Department of Chemistry, and the Molecular Biology Institute, University of California, Los Angeles, CA 90024, USA

Received 7 April 1981

ABSTRACT

A library of bacteriophage λ clones containing chicken chromosomal DNA was screened, using the adult β-globin cDNA plasmid pHb 1001 as a probe. Sixteen overlapping clones were isolated containing 35 kilobase pairs (kbp) of chicken DNA. Characterization of these clones revealed four β-like globin genes, some genomically repeated sequences, but no pseudo-genes. The four β-like genes have an average intergenic distance of less than 3 kbp, which is less than half of that found for the mammalian β-like globin gene clusters so far characterized. The overall features of the map were confirmed by genomic Southern analysis. Frequent deletions were shown to occur between the various β-like globin genes during phage propagation. The presumptive hatching gene in particular was always associated with abnormal λ clones although we were able to find one such clone that did contain a normal copy of the hatching gene itself. Probably such deletions explain the failure to recover this gene in previous attempts.

INTRODUCTION

The chicken β-like globin genes are a linked family of genes known to be differentially expressed during development. The isolation of recombinant bacteriophage containing the complete set of β-like globin genes is of obvious advantage in unraveling the complex regulatory events that occur as chicken embryos switch from embryonic to adult β-globin expression. While the entire β-like globin gene clusters from human,[1] rabbit,[2] and mouse[3,4] have been successfully isolated and mapped, complete recovery of the chicken β-like globin gene region has so far proved elusive.[5,6] In this paper, we report the isolation of the complete chicken β-like globin gene cluster as a set of overlapping recombinant phage.

Our analysis of these clones indicates that deletions frequently exist between the closely spaced hatching and adult β-globin genes. Frequent deletions during recombinant phage propagation could explain earlier failures to obtain recombinant phage containing the entire β-globin region.[5,6] Since closely spaced repeated sequences are not uncommon in euka-

ryotic genomes, appropriate measures should be taken to minimize deletions during the propagation of recombinant phage if homologous tandem, or near tandem, repeats are to be recovered intact.

MATERIALS AND METHODS

Isolation of Chicken β-like Globin Gene Recombinants from a Chicken DNA-λ Charon 4A Library. We obtained a library of chicken DNA fragments cloned in the λ vector charon 4A from Larry Souza and Marcel Baluda.[7] All subsequent work with these clones was carried out under P2+EK2 physical and biological containment. Approximately 300,000 plaque-forming units from this library were screened, using a modification of the Benton-Davis procedure.[8] Nitrocellulose filters (Millipore) were adsorbed on 150 mm petri plates for 2 min, treated with 1.5 M NaCl, 0.5 M NaOH for 30 sec, and then 3 M NaCl, 0.5 M Tris-HCl (pH 7.0) for 20 sec, and finally baked for 3 hr. Filters were prehybridized in 50% formamide, 0.75 M NaCl, 0.075 M sodium citrate, 3X Denhardt's solution,[9] 50 mM $NaHPO_4$ (pH 6.5), and 250 μg/ml denatured salmon sperm DNA for >1 hour at 42°. Hybridization was for 16-18 hours at 42° in 50% formamide, 0.75 M NaCl, 0.075 M sodium citrate, 1X Denhardt's solution, 20 mM $NaHPO_4$ (pH 6.5), 125 μg/ml denatured salmon sperm DNA, and 4 μC/ml of the ^{32}P-labeled large globin DNA Hpa II fragment from the adult β-globin cDNA plasmid pHb 1001.[10] After hybridization, the filters were washed twice with shaking in 0.3M NaCl, 0.03 M sodium citrate, 0.1% $NaDodSO_4$ for 1 hr at 67°, and once with 0.15 M NaCl, 0.015 M sodium citrate, 0.1% $NaDodSO_4$ for 30 min at 67°. The filters were then covered with Saran Wrap and placed against DuPont Cronex film in the presence of intensifying screens for 1-2 days at -70°.

Preparation of Phage DNA. 3×10^7 plaque-forming units of phage were incubated at 37° for 10 min with 3×10^8 DP50 SupF cells in standard Bacto-tryptone, yeast extract, NaCl medium containing diaminopimelic acid, thymidine and 10 mM $MgCl_2$. After incubation with shaking for 14 hours, 15 mM $MgSO_4$ was added, and the cell debris was centrifuged out. The supernatant was removed, and 35 g of NaCl was added along with 80 g of polyethylene glycol. The phage were allowed to precipitate overnight at 4° and then centrifuged. The precipitate was resuspended in 50 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$ and digested for 60 min at 37° with 10 μg/ml DNase I and 100 μg/ml RNase A. After centrifuging to remove insoluble debris, the phage were chloroform extracted once and then banded in a CsCl gradient. The phage band was removed from the tube by side puncture and dialysed into 50 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$ for 3 hours. The dialysate was made 20 mM in EDTA and 0.2% in $NaDodSO_4$ and then

digested for 60 min at 60° with 200 µg/ml protease K. The DNA was then extracted 3 to 4 times with phenol:chloroform (1:1) and dialysed overnight into 10 mM Tris-HCl (pH 7.4), 1 mM EDTA.

Restriction Map Analysis, Southern Blotting, and Electrophoretic Transfer. Restriction enzymes were obtained from Bethesda Research Labs or from New England Biolabs. 0.8 µg of recombinant phage DNA was digested under conditions recommended by the manufacturer and electrophoresed on 1% agarose (Sigma, Type II) slab gels in a buffer containing 40 mM Tris-HCl (pH 7.4), 20 mM sodium acetate, 2 mM EDTA, and 0.5 µg/ml ethidium bromide. The gels were visualized using short wavelength UV light, photographed, and transferred to nitrocellulose filters (Millipore) by the procedure of Southern[11] or to DPT paper[38] by electrophoretic transfer.[12] The transfer papers were hybridized to various probes and washed as described above. Restriction maps were obtained by electrophoresis of single, double, or triple restriction digests on 1% agarose gels and calibrating with size standards of Eco R1 digested wild-type λ (Bethesda Research) and Hae III digested M13 mp2.[13] A more detailed analysis of the various λ clones was obtained by isolating particular DNA fragments for further analysis from singly or doubly restricted recombinant phage DNA. The desired DNA fragments were excised from 0.7% agarose slab gels; and after dissolving the agarose in 3 volumes of 8 M $NaClO_4$, the DNA was bound to GF/C filters.[14] The isolated fragments were then either radioactively labeled for use as hybridization probes on Southern blots or further restricted with Hae III, Hpa II, or Hinf I and electrophoresed on tris-borate 6% polyacrylamide gels along with Hae III digested M13 mp2 as a size marker.

Preparation of ^{32}P-labeled DNA. To isolate a chicken adult β-globin probe, plasmid pHb 1001 DNA was restricted with Hpa II and electrophoresed preparatively on a tris-borate 6% polyacrylamide gel. A 466 bp DNA fragment containing most of the coding sequence for adult chicken β-globin[15] was excised and eluted, using the method of Maxam and Gilbert.[16] This and other DNA fragments prepared similarly were nick-translated[17] in 30 µl, using α-^{32}P-dCTP (600 Ci/mmole) from New England Nuclear, DNase I from Sigma, and E. coli DNA polymerase I from New England Biolabs. The nick-translation was stopped by adding EDTA to 10 mM, NaCl to 100 mM, $NaDodSO_4$ to 0.1%, and protease K (E. Merk) to 100 µg/ml, and incubating at 60° for 1 hr. The DNA was then extracted with phenol-chloroform twice and passed over a 1 ml Sephadex G50 Penefsky[18] column equilibrated with 10 mM Tris-HCl (pH 7.4), 1 mM EDTA. Specific activities were normally 1-5 x 10^8 cpm/µg.

RESULTS

Isolation and Characterization of β-like Globin Gene Clones. We have screened a chicken genomic λ library for recombinants containing β-like globin DNA, using as a probe the adult β-globin segment of the cDNA plasmid pHb 1001. This λ library was originally prepared by Souza et al[7] from size fractionated (16-22 kbp) partially Eco RI digested chicken DNA. From a screen of 300,000 plaques, 16 separate recombinant bacteriophage were isolated which hybridized to our β-globin probe. When the DNA from these recombinants was digested with Eco RI and the digestion products run on 1% agarose gels, it was apparent that the recombinants fell into six classes (data not shown). As a first step in characterizing the recombinant clones, we prepared a detailed restriction map for a member of each class, using the restriction enzymes Hind III, Eco RI, and Bam HI (see Materials and Methods for details). Restriction fragments from the various recombinant phage were sized on agarose gels (e.g., Fig. 1A), and Southern blots of these gels were probed with labeled β-globin DNA to localize the β-like globin structural sequences (e.g., Fig. 1B). Figure 2 summarizes the restriction map and globin gene localization data that were obtained.

In comparing our globin mapping data (Fig. 2) with that found by other workers,[5,6] we were surprised to find that the number of different globin containing restriction fragments was larger than expected and was, thus, in apparent conflict with the previous results. Either genetic polymorphism[19,20] or recombination during phage propagation[1,21-24] could account for the discrepancies between our data and the other studies. To test whether some type of genetic polymorphism was responsible for our results, the particular DNA (21710 myeloblast DNA) which was used to prepare the chicken recombinant library was obtained from Larry Souza, digested with Eco RI, run on an agarose gel, blotted onto nitrocellulose paper, and hybridized to the labeled β-globin probe (see lane 3 in Fig. 3A). For comparison, a mixture of DNAs from 20 separate chickens was Eco RI digested and run in lane 1 of Figure 3A. As further controls, identical samples of the Eco RI digested 21710 myeloblast DNA were coelectrophoresed with minute amounts of admixed Eco RI cut λCBGv6 (lane 2) or λCBGv4 DNA (lane 4). The results from Figure 3A show that the 13.3 kbp globin Eco RI fragment characteristic of λCBGv6 and the 1.9 kbp globin Eco RI fragment characteristic of λCBGv4 appear only in those lanes (2 and 4 respectively) containing the admixed cloned DNAs and not in lanes 1 and 3 which contain chicken genomic DNA alone. In other genomic blots (Fig. 3B), Bam Hl digestion of 21710 myeloblast DNA generated

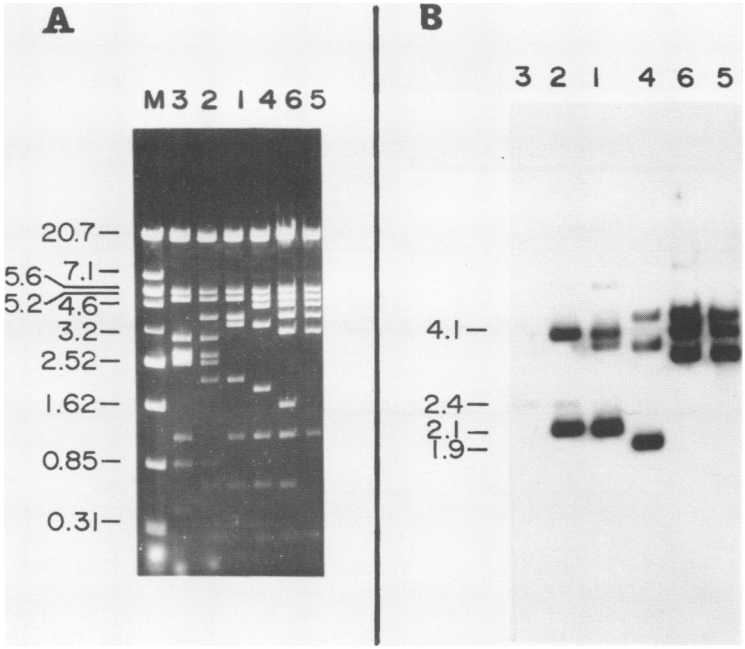

Figure 1. Agarose gel and blot hybridization analysis of recombinant phage DNA. (A) DNA from each clone was digested with restriction enzymes Eco RI and Hind III, electrophoresed on a 1% agarose gel, stained with ethidium bromide, and then visualized with short wavelength UV light. The far left marker contains a mixture of Eco RI restricted λ wild type and Hae III restricted M13 mp2 replicative form DNAs. (B) The DNA from the gel was blotted onto nitrocellulose filter paper and hybridized to a nick-translated DNA fragment from the adult β-globin cDNA plasmid, pHb 1001 (see Materials and Methods). The filter was washed and exposed to x-ray film with the aid of an intensifying screen for 16 hours. As we will show, the only globin sequence in λCBGv3 is from the third exon of the embryonic ε-globin gene so cross-hybridization to the labeled adult probe is weak in lane 3.

the same four β-globin containing fragments as have been reported previously.[6] We conclude that genetic polymorphism cannot account for the multiplicity of globin Eco RI fragment sizes in the cloned DNAs.

In view of the above, the most likely explanation for the appearance of the aberrant β-globin containing Eco RI bands is recombination during propagation of the phage in the bacterial host. Discrete deletions during the propagation of recombinant phage are known to occur for mammalian globin,[1,21] as well as non-globin[22-24] phage recombinants. Considering the extensive homology among the coding sequences of the chicken β-like globin genes and their

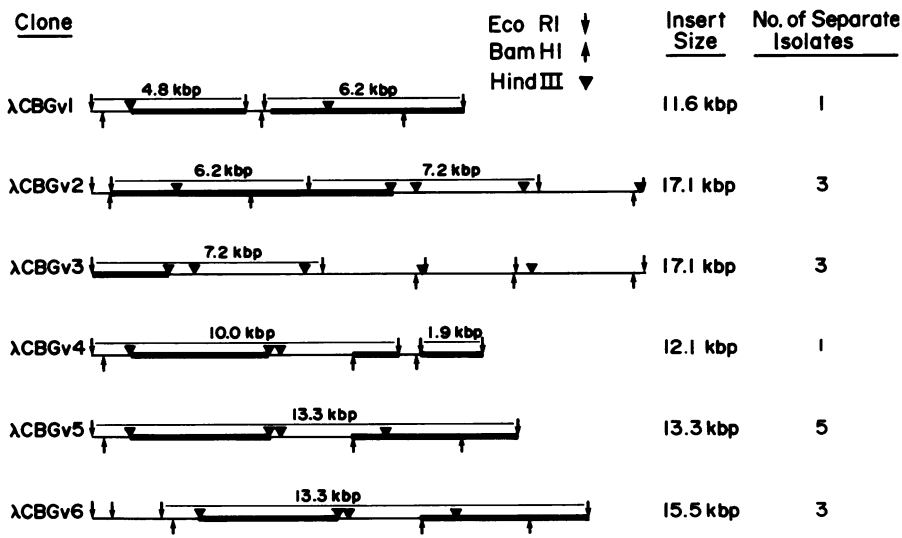

Figure 2. Restriction maps of the recombinant clones. The locations of the fragments which hybridize to the adult β-globin probe are indicated by thick black lines. The size of each Eco RI fragment containing β-like globin sequences is given above the fragment. The far right lane gives the number of separate isolates obtained for each clone. Of course, while each different clone is clearly of independent origin, we cannot exclude that some of the identical isolates have a common origin resulting from library amplification. The library was amplified once on plates (Larry Souza, personal communication). The second column from the right gives the size of the chicken DNA insert contained in each recombinant phage.

closely spaced tandem arrangement, one might expect frequent deletions of the DNA between globin genes. We shall show below that this is, in fact, the case.

Linkage Arrangement of the Four β-like Globin Genes and Characterization of the Deleted Regions in the Cloned DNA. Figure 2 shows that the chicken DNA segments in most of the clones are shorter than the 16-22 kbp fragment size employed in the original cloning.[7] The simplest explanation for the inordinate complexity of the Figure 2 mapping data is, therefore, that individual clones contain different deletions in their chicken DNA inserts. Based on this assumption, we have been able to link up the six recombinant clones into an overlapping set (Fig. 4) which contains the complete complement of known chicken β-like globin genes and which is consistent with the genomic Southern data of Figure 3.

To confirm the linkage arrangement and proposed deletions shown in Figure 4, we have conducted a series of cross hybridization experiments. DNA from

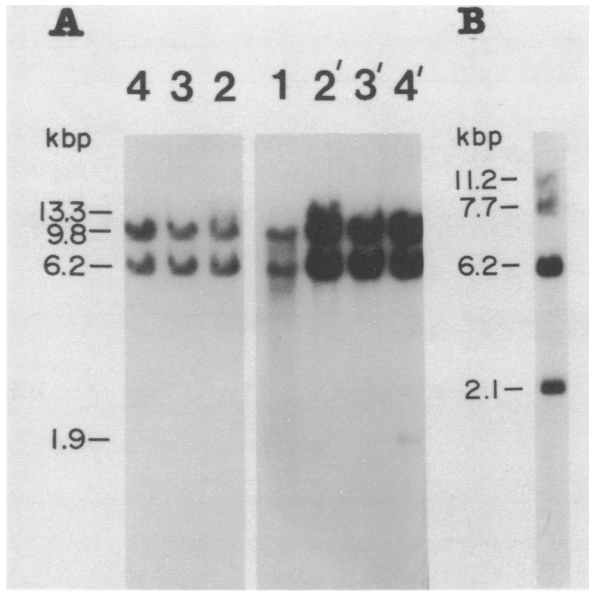

Figure 3. Genomic blots of the DNA used to prepare the chicken library of recombinant phage. (A) Various chicken DNA samples were restricted with Eco RI, electrophoresed on a 1% agarose gel, and blotted to nitrocellulose. The blot was then hybridized to the 466 bp Hpa II adult β-globin DNA fragment from pHb 1001, washed, and autoradiographed. The DNA samples are (1) 10 μg of DNA from 13-day chicken red blood cells which were pooled from 20 chickens, (2) 10 μg of 21710 myeloblast DNA (from which the chicken gene library was prepared) plus 1 pg of λCBGv6, (3) 10 μg of 21710 myeloblast DNA, (4) 10 μg of 21710 myeloblast DNA plus 1 pg of λCBGv4 DNA. Lanes 2,3,4 and 2',3',4' are identical except that autoradiographic exposure times were 2 days and 4 days respectively. (B) Southern blot autoradiograph of Bam HI digested 21710 myeloblast DNA.

each clone was digested with two or three restriction endonucleases, electrophoresed on an agarose gel, blotted onto nitrocellulose, and hybridized to a ^{32}P-labeled restriction fragment isolated from one of the recombinant clones. In Figure 5A, a 0.85 kbp Bam HI-Hind III DNA probe from the 5' end of clone λCBGv4 hybridized to the 0.85 kbp fragments in clones λCBGv1, λCBGv4, λCBGv5, and λCBGv6, but not to any of the fragments in clones λCBGv2 and λCBGv3, which do not extend into this region of the map. Thus, Figure 5A confirms the 5' lineup of the various recombinants.

The map in Figure 4 predicts that clones λCBGv4, λCBGv1, and λCBGv2 should all contain a 600 bp Eco RI fragment 3' to the putative hatching gene, while this Eco RI fragment should be absent in clone λCBGv5 or λCBGv6. Figure 5B

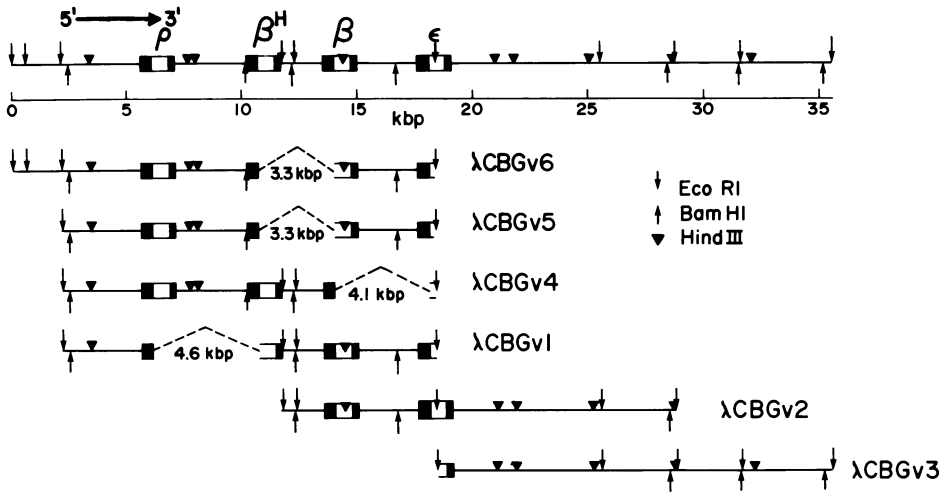

Figure 4. Linkage arrangement of the six overlapping clones containing four chicken β-like globin genes. The six independent clones have been arranged to show their overlapping sequences and their deleted regions. The deletion as shown for λCBGv4 is based on direct mapping data (see Fig. 7). The deletions in the other clones are drawn similarly for simplicity. The boxes indicate the approximate positions of the globin coding (black) and large intervening (white) sequences. These positions and the direction of transcription were determined partly from the published data of others[5,6,33] and partly from our own unpublished work. The genes were identified as follows, based in part on the characterization by others of independently isolated genomic clones.[5,33] The β gene has been shown to have Hpa II restriction sites matching those of an adult β cDNA clone.[33] We have confirmed this (Fig. 7 and unpublished results) for Hpa II as well as for Hae III, Hha I, and Hinf I by comparison to the cDNA sequence of Richards et al.[15] The ρ and ε genes were first shown to be embryonic by Dodgson et al[5] and Stalder et al,[6] and we have confirmed this in experiments which show that *in vitro* nuclear transcripts from 12-day embryonic red blood cells hybridize to a β but not to an ε intron probe (Landes and Martinson, in preparation). Recently, Dolan et al[30] have positively identified these genes by partial sequence analysis. The β"H" gene in our map has been reported to be adult-like,[6] and we have confirmed this in experiments which show that *in vitro* nuclear transcripts from 12-day embryonic red blood cell nuclei hybridize better than transcripts from 5-day nuclei to the Bam HI restriction fragments containing this and the β gene (unpublished results). However, our Hpa II, Hae III, Hha I and Hinf I restriction maps (unpublished) show that the gene designated β"H" in our map, unlike the β gene, does not match the cDNA sequence for β globin.[15] By elimination, we conclude that β"H"in our map is most probably the gene for the β-like chain of "hatching hemoglobin."[34-36]

demonstrates that the 600 bp Eco RI fragment isolated from clone λCBGvl hybridizes to a 600 bp Eco RI fragment in clones λCBGvl, λCBGv2, and λCBGv4, but does not hybridize to any restriction fragment in clone λCBGv6. Thus, the

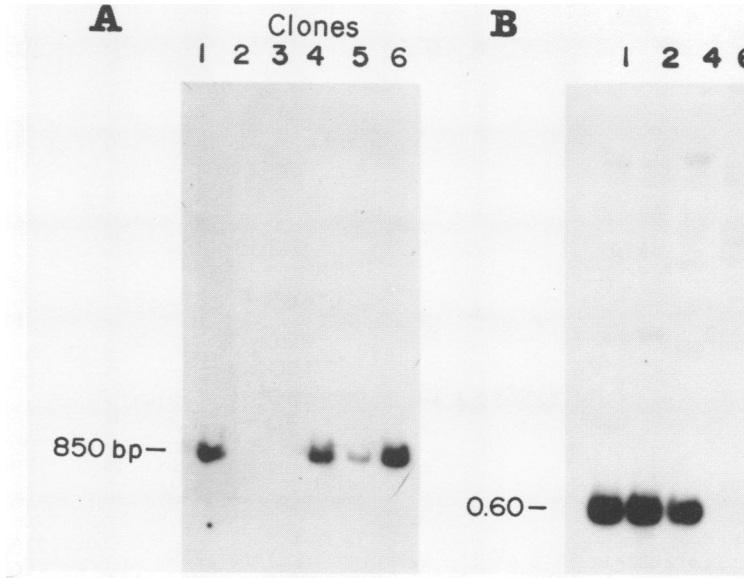

Figure 5. Hybridization data demonstrating sequence overlap among the various clones. (A) DNA from each of the six clones was digested with Eco RI, Hind III, and Bam HI and fractionated on a 1% agarose gel. The Southern blot of this gel was hybridized to a labeled 850 bp Bam HI-Hind III fragment from the 5' end of the chicken insert of clone λCBGv4 and autoradiographed. (B) DNA from clones λCBGv1, λCBGv2, λCBGv4, and λCBGv6 was electrophoresed on a 1% agarose gel. The blot of the gel was hybridized to the labeled 600 bp β"H"-β intergenic Eco RI fragment from clone λCBGv1 and autoradiographed.

results from Figure 5B are consistent with the prediction from the linkage map.

To confirm further the map in Figure 4, an additional cross-hybridization was carried out using an intron probe from the ε gene. DNA from clones λCBGv1, λCBGv2, λCBGv4, and λCBGv5 was digested to completion with Eco RI, Hind III, and Bam HI and then digested further, but only partially, with Hinf I. The restricted DNA was fractionated on an agarose gel and then electrophoretically transferred to DPT paper. As probe, a 280 bp Hae III-Eco RI restriction fragment representing the 5' third of the ε gene major intron was used. When hybridized to the DPT paper, this probe will label only restriction fragments whose 3'termini are the Eco RI site of the ε intron. Figure 6A shows the ordered ladder of partial Hinf I fragments which is detected. The sizes of the fragments reflect the distances of individual Hinf I sites to the left of

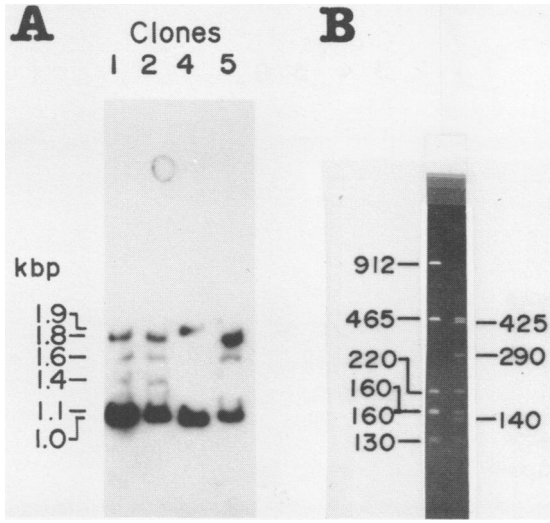

Figure 6. Characterization of the deletion in clone λCBGv4. (A) DNA from each of clones λCBGv1, λCBGv2, λCBGv4, and λCBGv5 was digested with Eco RI, Hind III, and Bam HI and ethanol precipitated. DNA from each clone was then resuspended in Hinf I digestion buffer, partially digested with Hinf I, fractionated on a 1.4% agarose gel, and electrophoretically transferred to APT paper. The transfer was hybridized to a nick-translated Hae III-Eco RI fragment corresponding to the 5' third of the ε intron. This fragment was obtained from a pBR322 subclone of a Hae III fragment originally obtained from λCBGv2. That the 1.0 kbp fragment of clone 4 does indeed migrate slightly ahead of the 1.1 kbp fragments of the other clones was confirmed in a shorter exposure of the autoradiogram. (B) The 2.1 kbp Bam HI-Hind III fragment from the 5' end of the insert in clone λCBGv2 (left lane) and the 1.9 kbp Eco RI fragment from the 3' end of the insert in clone λCBGv4 (right lane) were isolated from agarose gels, digested to the limit with Hpa II, and fractionated on a 6% polyacrylamide gel. The base pair sizes of the resulting fragments are shown in the margin. (See Fig. 7).

the Eco RI site in the ε intron. Two principal conclusions can be drawn from Figure 6A. First, it can be seen that the DNA from all four clones hybridizes to the ε gene intron probe. Thus, each of the four clones contains at least a portion of the ε intron. Second, it can be seen that the hybridization patterns for clones λCBGv1, λCBGv2, and λCBGv5 are similar; whereas that for λCBGv4 differs, reflecting its 4.1 kbp deletion (see below).

The only λ isolate containing an apparently accurate copy of the genomic $\beta^{"H"}$ gene is λCBGv4. In order to confirm that the 4.1 kbp deletion in this clone does not fall within the $\beta^{"H"}$ gene or its flanking regions, we have isolated the 1.9 kbp Eco RI fragment of this clone and prepared a map of the Hae

III, Hha I, Hinf I, and Hpa II restriction sites (Figs. 6 and 7). Comparison of this restriction map with that of appropriate segments from clone λCBGv2 indicates that the end of the deletion in clone λCBGv4 maps within about 70 bp of the 3' end of the middle β exon (Fig. 7). Consistent with this restriction analysis we regularly observe (e.g. Fig.1) that the adult β probe, pHb 1001, hybridizes better to the 1.9 kbp Eco RI fragment from clone λCBGv4 than it does to the embryonic ρ gene-containing fragment from the same clone. Conversely, the hybridization data of Figure 6A show that the 3' end of this same 1.9 kbp fragment of λCBGv4 is homologous to the large intron of the ε gene. Therefore, the 1.9 kbp fragment contains both adult and embryonic sequences and we conclude that the 4.1 kbp deletion of clone λCBGv4 results from cross-over between the β and ε globin genes within their middle exons and does not involve a perturbation of the β"H" gene or its flanking sequences.

Localization of Repetitive Sequences. Repetitive sequences have been found to be interspersed with unique genes in a number of species.[25-28] To determine which regions within the chicken β-like globin gene cluster contain repetitive sequences, we have ^{32}P-labeled total chicken red blood cell DNA and hybridized this to our cloned DNA under conditions (i.e., normal Southern hybridization conditions) which allow only abundant sequences to anneal. Recombinant phage λCBGv2, λCBGv3, and λCBGv4 were digested with two or three restriction enzymes, fractionated on an agarose gel, and transferred to DPT paper. The transfer was then hybridized to the labeled total chicken DNA, washed, and autoradiographed. Figure 8 demonstrates that the following regions contain repetitive DNA: (a) the 4.4 kbp Hind III fragment, containing the ρ gene, and the intergenic 2.3 kbp Hind III-Bam HI fragment from between the ρ and β"H" genes; (b) the 2.4 kbp Eco RI-Hind III and the 3.3 kbp Hind III fragments on the 3' flanking side of the ε gene; and (c) the 3.1 kbp Hind III-Bam HI fragment located at the extreme 3' end of the map in Figure 4. This assay would not detect repetitive elements of low repetition frequency.[29]

DISCUSSION

We have characterized six overlapping recombinant clones which represent about 35 kbp of DNA containing the entire β-like globin gene region of chicken. The four β-like globin genes which we report here account for all restriction fragments which have been detected in genomic Southern blots probed with the adult β-globin cDNA clone, pHb 1001. Unless other highly divergent and

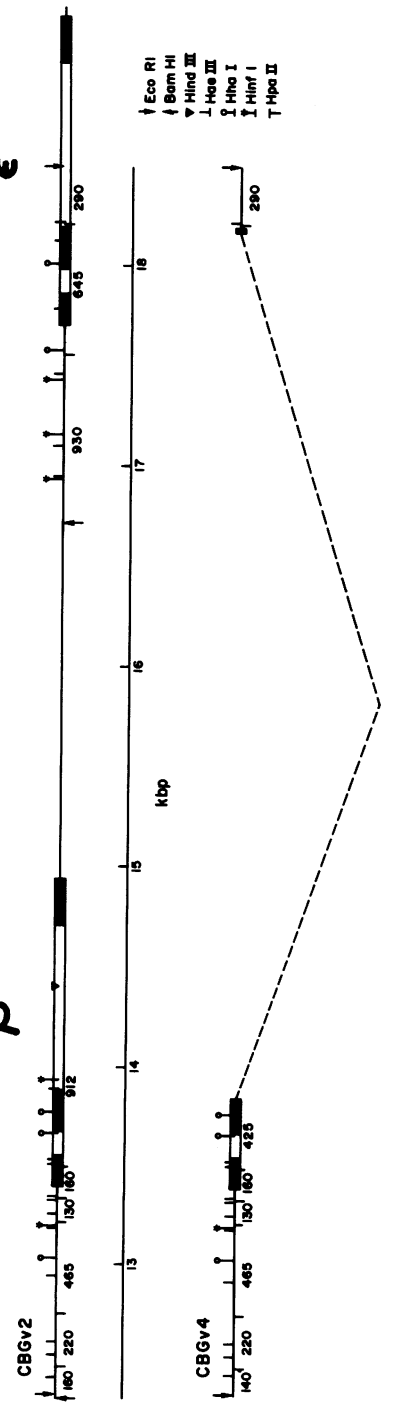

Figure 7. The map of the deletion in λCBGv4. Based on data such as that shown in Figure 6, a restriction map for Hae III, Hha I, Hinf I, and Hpa II sites was generated for appropriate overlapping regions of clones λCBGv2 and λCBGv4. (We should point out that we find some Hinf I sites to be highly favored over others. Only the most preferred sites are shown within the regions mapped.) The mapping data show that the deletion in clone λCBGv4 must start within 70 bps of the 3' end of the second exon in the β-globin gene and terminate at a similar position within the second exon of the ε-globin gene. Note that, unlike in Figure 4, we show the locations of both the major and the minor introns in this figure. By comparison of our restriction map with the β globin cDNA sequence of Richards et al,[15] we deduce the 5' and 3' boundaries of the second exon to be situated as shown with respect to a central Hha I site. These locations are consistent with intron interventions between codons 30 & 31 and 104 & 105 as for mammals.[37] We have drawn the small intron to be about 125 bp by analogy to mammals,[37] and the large intron length is shown as 820 bp.[5,33]

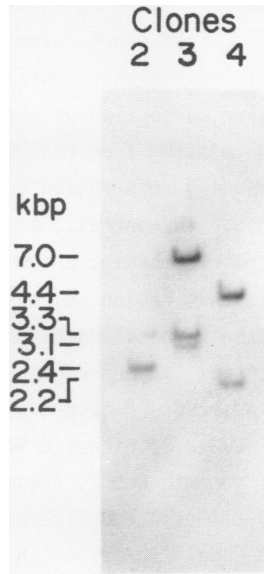

Figure 8. Hybridization of repetitive DNA. Clones λCBGv2 and λCBGv4 were digested with Eco RI, Bam HI, and Hind III. Clone λCBGv3 was digested with Bam HI and Hind III. After fractionation on a 1% agarose gel, the fragments were transferred to APT paper and hybridized to 3 x 10^8 cpm of nick-translated 13-day embryonic red blood cell DNA (3 x 10^8 cpm/μg) in 5 ml of hybridization buffer. The transfer was washed and then autoradiographed for 5.5 hours.

as yet uncharacterized β-like globins exist, our cloned DNA sequences include the entire chicken β-like globin gene cluster.

Given the linkage map in Figure 4, it is possible to compare the average separation between the β-like genes of chickens to that found in mammals.[1-4] The mouse β-like globin gene cluster contains 7 genes and pseudogenes in >47 kbp with the average intergenic distance being 6.3 kbp. The rabbit β-like globin gene cluster contains four genes in 25.5 kbp with an average intergenic distance of 6.6 kbp. The human β-like globin gene cluster is the largest (53 kbp), containing 5 genes and 2 pseudogenes and, in this case, the average intergenic distance (including the distances between pseudogenes) is 6.9 kbp. In contrast, the chicken β-like globin gene cluster which contains four genes in 14 kbp, has an average intergenic separation of only 2.8 kbp. Thus, the chicken β-like globin genes have much less intergenic flanking sequences than do those of the human, rabbit, or mouse. Therefore, the chicken β-like globin gene region may be particularly useful for investi-

gating the roles of intergenic DNA (including repetitive sequences) in the developmental regulation of the β-like globin genes.

Previous workers have isolated λ clones containing three of the chicken β-like globin genes.[5,6] However, a portion of the $\beta^{"H"}$-globin gene and its 3' flanking region appear to be missing from the Dodgson-Engel library.[6,30] All 10 of our phage isolates spanning this region contain deletions, most often (8 out of 10) between $\beta^{"H"}$ and β. In contrast, among the six isolates not spanning this region, all of the recombinant phage were undeleted and stable during further passage. Why is this region so consistently associated with deletion artifacts? It is unlikely that length constraints introduced by the use of an Eco RI partial digest for library construction are responsible in some way for the deletions we observe. Figure 4 shows that both deleted (λCBGv1,4,5,6) and undeleted (λCBGv2,3) clones originated during construction of the library by insertion of genomic Eco RI fragments of similar size (16.7-18.3 kbp for the deleted clones; 17.0-17.2 kbp for the undeleted clones). Moreover the Dodgson-Engel library which has lost the most deletion-prone region (see above) was not constructed by the partial Eco RI method. Nor is it likely that the deletions which we have found have arisen because the regions are highly deleterious to growth in E. coli. Each of the regions, which are deleted in some phage, are recovered intact and remain stable during passage in other recombinants. We believe the high tendency to delete between $\beta^{"H"}$ and β most likely reflects a high degree of homology between the coding regions of these genes.[30] Their close proximity in the genome probably leads to high frequency intramolecular recombination and consequent deletion in λ. The apparently high pressure towards deletion argues against an intermolecular mechanism which should produce duplications as well as deletions.[31]

If the $\beta^{"H"}$-β region is so hyper-deletable, how is it that we have, in fact, recovered it at all in two instances (λCBGv1 and λCBGv4)? A pertinent observation is that both of these clones have *flanking* deletions in the chicken DNA. Presumably there is a tendency, albeit somewhat less, of the ρ-$\beta^{"H"}$ and the β-ε regions also to undergo deletion. If such an event occurs in λ prior to the $\beta^{"H"}$-β deletion, as apparently it did in both λCBGv1 and λCBGv4, further deletion of the $\beta^{"H"}$-β region would render the already shortened phage DNA even shorter. The resulting doubly deleted molecules would be too short to be packaged efficiently and, therefore, would not be recovered as phage.[31,32] Consistent with this explanation is the observation that λCBGv4 can be passaged stably but is recovered in several fold lower yield than λCBGv2, 3, 5, or 6.

The above findings suggest that for recovery of certain types of DNA in λ, *in vitro* packaging may preferably be carried out using somewhat smaller fragments of foreign DNA (e.g., 12-15 kbp).

The isolation of λCBGv4 is fortunate because it is the only clone containing the $\beta^{"H"}$ intron and 3' flanking DNA. The availability of these regions in cloned form is essential for studies directed at a complete description of the developmental regulation of transcription of the chicken β-like globin gene cluster. Our restriction mapping places the deletion within the β gene of λCBGv4. Therefore, we can be confident that the $\beta^{"H"}$ gene and all $\beta^{"H"}$-β intergenic DNA is normal in this clone and reflects the true genomic arrangement.

ACKNOWLEDGEMENTS

We thank Drs. Marcel Baluda and Larry Souza for their gift of the λ library, Dr. Winston Salser for the gift of pHb 1001, Dr. Doug Engel for a preprint of reference 30 and Dr. Greg Landes for sub-clones from the λ isolates and for valuable discussions. This work was supported by grants from the California Institute for Cancer Research and the University of California Cancer Research Coordinating Committee.

REFERENCES

1. Fritsch, E.F., Lawn, R.M. and Maniatis, T. (1980) Cell 19, 959-972.
2. Lacy, E., Hardison, R.C., Quon, D. and Maniatis, T. (1979) Cell 18, 1273-1283.
3. Jahn, C.L., Hutchison III, C.A., Phillips, S.J., Weaver, S., Haigwood, N.L., Voliva, C.F. and Edgell, M.H. (1980) Cell 21, 159-168.
4. Leder, P., Hansen, N.J., Konkel, D., Leder, A., Nishioka, Y. and Talkington, C. (1980) Science 209, 1336-1342.
5. Dodgson, J.B., Strommer, J. and Engel, J.D. (1979) Cell 17, 879-887.
6. Stalder, J., Larsen, A., Engel, J.D., Donal, M., Groudine, M. and Weintraub, H. (1980) Cell 20, 451-460.
7. Souza, L.M., Komaromy, M.C. and Baluda, M.A. (1980) Proc. Natl. Acad. Sci. 77, 3004-3008.
8. Benton, W.D. and Davis, R.W. (1977) Science 196, 180-182.
9. Denhart, D.T. (1966) Biochem. and Biophys. Res. Comm. 23, 641-646.
10. Salser, W.A., Cummings, I., Liu, A., Strommer, J., Padayatty, J. and Clarke, P. (1979) in Cellular and Molecular Regulation of Hemoglobin Switching, Stamatoyannopoulos, G. and Nienhuis, A. Eds., pp. 621-643, Grune and Stratton, New York.
11. Southern, E.M. (1975) J. Mol. Biol. 98, 503-517.
12. Bittner, M., Kupferer, P. and Morris, C.F. (1980) Anal. Biochem. 102, 459-471.
13. Gronenborn, B. and Messing, J. (1978) Nature 272, 375-377.
14. Chen, C.W. and Thomas, Jr., C.A. (1980) Anal. Biochem. 101, 339-341.

15. Richards, R.I., Shine, J., Ullrich, A., Wells, J.R.E. and Goodman, H.M. (1979) Nucleic Acids Res. 7, 1137-1147.
16. Maxam, A.M. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. 74, 560-564.
17. Rigby, P.J.W., Dieckmann, M., Rhodes, C. and Berg, P. (1977) J. Mol. Biol. 113, 237-251.
18. Penefsky, H.S. (1977) J. Biol. Chem. 252, 2891-2899.
19. Weinstock, R., Sweet, R., Weiss, M., Cedar, H. and Axel, R. (1978) Proc. Natl. Acad. Sci. 75, 1299-1303.
20. Lai, E.C., Woo, S.L.C., Dugaiczyk, A. and O'Malley, B.W. (1979) Cell 16, 201-211.
21. Lauer, J., Shen, C-K. J. and Maniatis, T. (1980) Cell 20, 119-130.
22. Tronick, S.R., Robbins, K.C., Canaani, E., Devare, S.G., Andersen, P.R. and Aaronson, S.A. (1979) Proc. Natl. Acad. Sci. 76, 6314-6318.
23. Carroll, D. and Ajioka, R.S. (1980) Gene 10, 273-281.
24. McClements, W.L., Enquist, L.W., Oskarsson, M., Sullivan, M. and Vande Woude, G.F. (1980) J. Virol. 35, 488-497.
25. Kaufman, R.E., Kretschmer, P.J., Adams, J.W., Coon, H.C., Anderson, W.F. and Nienhuis, A.W. (1980) Proc. Natl. Acad. Sci. 77, 4229-4233.
26. Fritsch, E.F., Lawn, R.M. and Maniatis, T. (1980) Cell 19, 959-972.
27. Hoeijmakers-van Dommelen, H.A.M., Grosveld, G.C., de Boer, E., Flavell, R.A., Varley, J.M. and Jeffreys, A.J. (1980) J. Mol. Biol. 140, 531-547.
28. Moore, G.P., Costantini, F.D., Posakony, J.W., Davidson, E.H. and Britten, R.J. (1980) Science 208, 1046-1048.
29. Wood, W.I., Hickol, J. and Felsenfeld, G. (1981) J. Biol. Chem. 256, 1502-1505.
30. Dolan, M., Sugarman, B.J., Dodgson, J.B. and Engel, J.D. (1981) Cell, in press.
31. Bellett, A.J.D., Busse, H.G. and Baldwin, R.L. (1971) in The Bacteriophage Lambda, Hershey, A.D. Ed., pp. 501-513, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. Blattner, F.R., Williams, B.G., Blechl, A.E., Denniston-Thompson, E., Faber, H.E., Furlong, L., Grunwald, D.J., Kiefer, D.O., Moore, D.D., Schumm, J.W., Sheldon, E.L. and Smithies, O. (1977) Science 196, 161-169.
33. Ginder, G.D., Wood, W.I. and Felsenfeld, G. (1979) J. Biol. Chem. 254, 8099-8102.
34. Brown, J.L. and Ingram, V.M. (1974) J. Biol. Chem. 249, 3960-3972.
35. Moss, B.A. and Hamilton, E.A. (1974) Biochim. Biophys. Acta 371, 379-391.
36. Keane, R.W. and Abbott, U.K. (1980) Develop. Biol. 75, 442-453.
37. Efstratiadis, A., Posakony, J.W., Maniatis, T., Lawn, R.M., O'Connell, C., Spritz, R.A., DeRiel, J.K., Forget, B.G., Weissman, S.M., Slightom, J.L., Blechl, A.E., Smithies, O., Baralle, F.E., Shoulders, C.C. and Proudfoot, N.J. (1980) Cell 21, 653-668.
38. Alwine, J.C., Kemp, D.J., Parker, B.A., Reiser, J., Renart, J., Stark, G.R. and Wahl, G.M. (1979). Methods Enzymol. 68, 220-242. (See Footnote 51)