Proc. Natl. Acad. Sci. USA
Vol. 77, No. 5, pp. 2596–2600, May 1980
Biochemistry

Analysis of the closely linked adult chicken α-globin genes in recombinant DNAs

(chromosomal linkage/intervening sequences/embryonic α-globin gene)

JAMES DOUGLAS ENGEL* AND JERRY B. DODGSON†

*Department of Biochemistry and Molecular Biology, Northwestern University, Evanston, Illinois 60201; and †Departments of Microbiology and Public Health and Biochemistry, Michigan State University, East Lansing, Michigan 48824

Communicated by David Shemin, February 19, 1980

ABSTRACT **Recombinant bacteriophage (from a library of chicken chromosomal DNA fragments inserted into λ Charon 4A) have been isolated which contain the coding information for both of the adult chicken α-globin genes, α^A and α^D. One of these recombinant phage also contains an as yet unidentified embryonic α-like globin gene sequence. The two adult genes are encoded on the same DNA strand and are separated by approximately 2.4 kilobase pairs, with the arrangement of the genes relative to the direction of transcription being 5′-α^D-α^A-3′. Electron microscopic R-loop visualization experiments demonstrate that both α-globin genes contain two intervening sequences of similar size in a manner analogous to the structure observed in the mouse α-globin gene. The linkage of the two highly divergent chicken adult α-globin genes further underscores the principle that chromosomal clustering of families of developmentally related genes may be a general phenomenon in higher eukaryotic gene sequence arrangement.**

Avian erythropoiesis involves the temporal, programmed expression of select α- and β-type globin genes during embryonic development. There appear to be two specific early embryonic α-type (π, π′) globins and two α-type (α^A, α^D) globins present in both adult and embryonic chicken red blood cells, as well as distinct embryonic and adult β-type globins (1). The temporal change in globin gene expression from the primitive or early embryonic pattern to the definitive (late embryonic and adult) pattern occurs between days 5 and 9 after fertilization (2, 3). The molecular mechanisms that regulate these ordered changes during embryonic development are unknown. In the hope of elucidating control functions involved in developmentally related gene expression, we have isolated and characterized the adult chicken α-globin genes.

By using recombinant DNA methodology (4–6) we have isolated several recombinant phage among which are contained the entire structural gene information of many of the genes coding for chicken globin mRNAs as well as their directly adjacent DNA sequences. This approach has led to the isolation of a number of recombinants that contain two or more globin genes within a relatively short span of chromosomal chicken DNA. In this paper, we demonstrate that the two adult chicken α-globin genes are closely linked to one another and to a strongly hybridizing embryonic α-globin locus and, thus, the sequence arrangement of the globin family in chickens is likely to be a clustering of either α- or β-type sequences, with both sets of genes residing on different chicken chromosomes (ref. 7 and unpublished observations).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Isolation of λCαG2 and λCαG5. Recombinant DNA bacteriophage were prepared from randomly sheared chicken chromosomal DNA and from λ Charon 4A DNAs as described (5). The phage library, consisting of about 5×10^5 unique recombinants, was screened, plaques were purified, and phage DNA was prepared as described (5). *Eco*RI-digested recombinant DNAs were blotted to nitrocellulose filters and then hybridized to ^{32}P-labeled pβG1 DNA (pRcβ1; adult rabbit β-globin cDNA plasmid recombinant; ref. 8), adult chicken globin cDNA, and embryonic chicken globin cDNA (5, 9). This preliminary screening procedure separated clones of embryonic and adult β-type globin sequence, adult α-globin sequences (those which hybridize to adult cDNA, but not to pβG1), and embryonic α-type globin sequences (those which hybridize to embryonic cDNA but not to adult cDNA or pβG1). Two of the recombinants fell into the second category. These recombinants have been designated λ Charon 4A Chicken α-Globin 2 and 5 (λCαG2 and λCαG5).

Restriction Enzyme Mapping. Phage λ recombinant DNA (0.6 μg) was digested to completion with commercial restriction endonucleases (New England BioLabs). The DNA was electrophoresed, stained with ethidium bromide, photographed, and blotted to nitrocellulose filters (5, 9). Adult or 5-day embryonic chicken globin cDNA (≈2.5×10^8 cpm/μg) was hybridized to the blot, and the restriction fragments containing globin complementary sequences were determined by autoradiography. For absolute verification of the relative position of ambiguous restriction enzyme sites within the mapping patterns, some recombinant DNA fragments produced by restriction enzyme digestion were subcloned into pBR322 plasmid vectors, which were then further mapped by multiple digests (data not shown) or by the partial digest procedure of Legerski *et al.* (10). DNA fragment sizes were determined by comparison with the relative migration of λ (*Eco*RI) (11, 12) or pBR322 digestion products (13) (or both) run as markers in outer lanes of the same gel.

Filter Hybridization and Washing. After blotting, filters were baked for 4 hr at 75°C under reduced pressure and then prehybridized for a minimum of 4 hr at 42°C with 50% (vol/vol) formamide, 1 M NaCl, 50 mM Tris·HCl (pH 8.2), 1 mM EDTA, 10× Denhardts' solution, 0.1% NaDodSO$_4$, 50 μg of heat-denatured, sonicated calf thymus DNA per ml, and 20 μg of poly(rA) per ml. This solution was poured out and replaced with identical fresh solution containing probe and hybridized for a minimum of 5 × (filter $C_0t_{1/2}$ or $D_0t_{1/2}$) (9). [$C_0t_{1/2}$ is the

Abbreviations: kbp, kilobase pairs; bp, base pairs; IVS, intragenic sequences not present in mature mRNA.

initial concentration of DNA (mol of nucleotide/liter) multiplied by time (sec) necessary for 50% hybridization.] Low-criterion washes differed from high-criterion washes (9) only by the addition of NaCl to 50 mM in the former. Filter hybrids formed with heterologous (i.e., rabbit globin) probes were all washed at low criterion, whereas homologous (i.e., chicken globin) probes were all washed at high criterion.

Electron Microscopy. R loops (14) were formed by hybridization of adult globin mRNA to trioxalen-crosslinked λCαG2 DNA as described (5, 15). The poly(A) tails in R loops were labeled by using poly(BrdU)-tailed pBR345 (gift of David Haback) as described (5, 15).

Miscellaneous. Rabbit globin mRNA and pβG1 plasmid DNA were gifts of Tom Maniatis (Caltech). Chicken globin mRNA and labeled cDNA were prepared as described (9).

Containment. Experiments involving chicken/λ Charon 4A recombinant bacteriophage were carried out as specified by the original and revised National Institutes of Health Guidelines for Recombinant DNA Research.

RESULTS

λCαG2 Contains Adult α-Globin Gene Sequences. Two chicken DNA recombinant bacteriophage, λCαG2 and λCαG5, were isolated as probable adult α-globin gene-containing clones. λCαG2 DNA was cleaved with *Eco*RI, electrophoresed, stained with ethidium bromide, photographed (Fig. 1, lane A), and blotted to nitrocellulose filter paper (9). The filter was successively hybridized to ^{32}P-labeled pβG1 DNA,

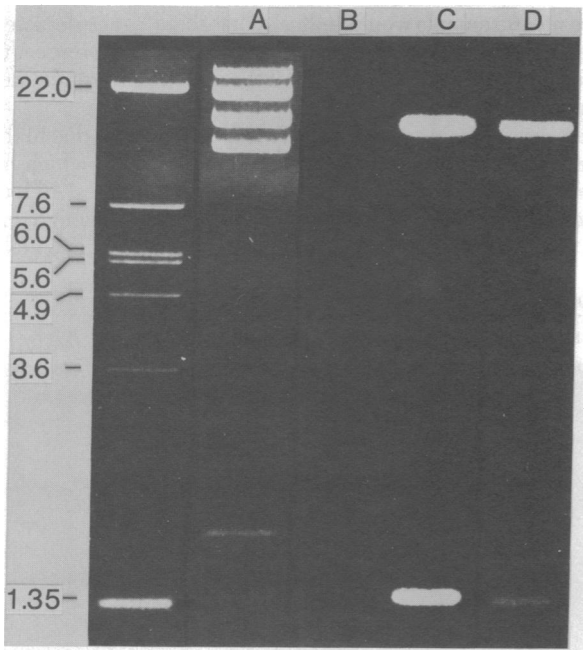

FIG. 1. λCαG2 contains the α^A and α^D (adult) chicken α-globin genes. λCαG2 DNA was cleaved with *Eco*RI, electrophoresed in a 0.7% vertical slab gel, stained with ethidium bromide, and photographed as described (5) (lane A; the highest M_r component is annealed left and right arms of λ Charon 4A vector). The DNA was then transferred to nitrocellulose and hybridized to ^{32}P-labeled pβG1 DNA (8) (lane B), adult chicken globin cDNA (lane C), and adult rabbit globin cDNA (lane D). The same filter was used for all hybridizations; probe was removed prior to initiating a new hybridization reaction by washing the filter twice in distilled H_2O at 60°C for 15 min. Exposure times were 3.5, 0.5, and 0.5 hr, respectively, reflecting the specific activity of the nick-translated (lane B) and cDNA (lanes C and D) probes (3×10^8, 2.5×10^9, and 4×10^9 dpm/μg, respectively). Size standardization markers (to the left of lane A) are λ/*Eco*RI (partial) (12) and the largest φX174/*Hae* III bands; sizes are given in kilobase pairs.

adult chicken globin cDNA, and adult rabbit globin cDNA. The hybridization pattern seen in Fig. 1 demonstrates the following. (*i*) Globin complementary sequences exist on both the 13.5- and 1.35-kilobase pair (kbp) *Eco*RI fragments. (*ii*) These sequences are not homologous to β-globin probes but are strongly homologous to adult chicken globin cDNA, thereby demonstrating that these are adult α-globin sequences. [We have repeated these experiments (not shown) with pHb1001 (adult chicken β-globin cDNA clone) (16) as probe instead of pβG1 with results identical to those in Fig. 1, lane B.] (*iii*) Adult rabbit globin cDNA has strong sequence homology to the 13.5-kbp *Eco*RI fragment, but very little homology to the 1.35-kbp *Eco*RI fragment of CαG2.

We have previously exploited the sequence homologies between rabbit and chicken globin sequences to identify certain restriction fragments containing chicken globin genes in chromosomal blots (9). In particular, the adult rabbit α-globin is highly homologous to the major adult chicken α-globin, α^A, but has very little homology to the minor adult gene, α^D (17, 18). This suggests that the 13.5-kbp *Eco*RI fragment contains the α^A (major) globin gene, whereas the 1.35-kbp *Eco*RI fragment of λCαG2 contains the entire α^D (minor) adult chicken globin gene (verified below and by fine structure mapping of subclones of λCαG2; unpublished observations). Determination of the DNA sequence is necessary for the absolute verification of identity of the two adult genes.

Restriction Enzyme Mapping of λCαG2 and λCαG5. Both λ recombinants were digested with several individual restriction enzymes (*Eco*RI, *Bam*HI, *Hin*dIII, and *Kpn* I) and with all paired combinations of these, electrophoresed, stained with ethidium bromide, photographed, and blotted to nitrocellulose (5). The filter was then hybridized to ^{32}P-labeled adult globin cDNA and exposed for autoradiography. By comparison of the pattern of DNA fragment labeling on the blots with the total DNA fragment pattern of the stained gel, the restriction enzyme cleavage maps of λCαG2 and λCαG5 were deduced (Fig. 2). Ambiguous cleavage sites were verified by further experiments (see *Materials and Methods*).

λCαG2 contains an insert of 16.8 kbp of chicken chromosomal DNA. This α-globin recombinant contains two separated DNA sequences which hybridize strongly to adult chicken globin cDNA. One is contained completely within the 1.35-kbp *Eco*RI fragment bounded by the right arm of λ Charon 4A (λR); the other is almost totally within the 1.5-kbp *Hin*dIII/*Kpn* I fragment located approximately 1.5 kbp away from the 1.35-kbp *Eco*RI fragment of Fig. 2*A*. The 1.4-kbp fragment separating the two regions of strong globin homology contains no regions with homology to the globin cDNA probes.

λCαG5 contains both α-globin genes in λCαG2, but extends the total chromosomal DNA segment studied some 8 kbp beyond the left edge of λCαG2 (shown below to be towards the 5′ direction, in the transcriptional sense, in the adult α-globin genes; Fig. 2*B*). No differences in the cleavage sites of any of the four restriction enzymes used in the mapping studies have been observed between λCαG2 and λCαG5 in their region of overlap.

When cDNA prepared from 5-day globin mRNA was hybridized to λCαG5, a labeling pattern was observed that was different from that with adult globin cDNA. Strong hybridization was observed to both of the previously identified sequences contained in λCαG2 (Fig. 3) but, in addition, very strong hybridization was also observed to a third region, some 2.3 kbp distal to α^D from α^A (Fig. 3). This sequence hybridized to embryonic, but not to adult, globin cDNA, and preliminary data suggest that this sequence also does not hybridize strongly to either of the chicken α-like clones pHb1003 or pHb1008

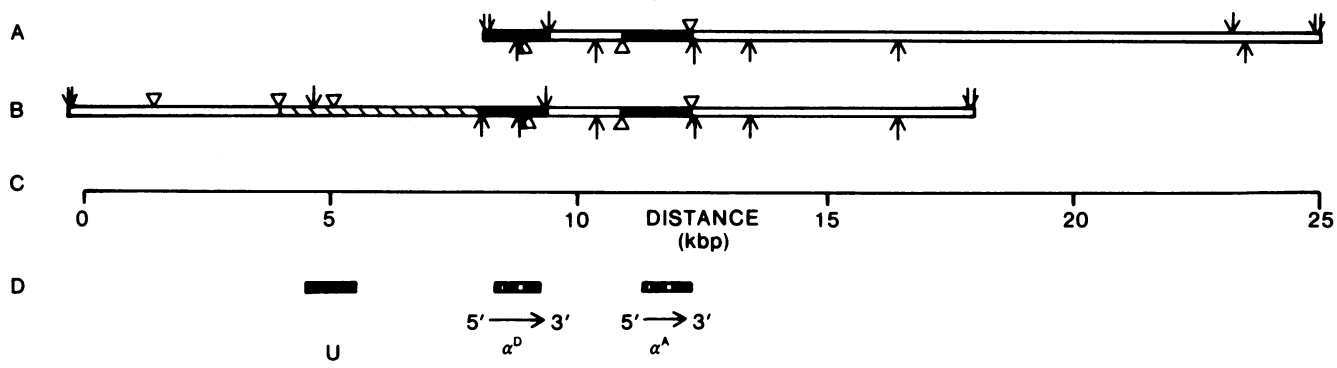

FIG. 2. Restriction maps and direction of transcription of the adult α-globin genes in λCαG2 and λCαG5. Restriction maps of λCαG2 and λCαG5 were deduced by described methods (4, 5, 10). Only the recombinant insert of the Charon 4A clones, flanked by synthetic *Eco*RI linkers, is shown. Regions of strong sequence homology to embryonic and adult chicken globin cDNA are shown as solid boxes; regions of strong homology only to embryonic cDNA are shown as diagonal lines. For both λCαG2 (*A*) and λCαG5 (*B*) the right linker on the diagram is joined to the left arm of λ Charon 4A (12). The entire region of this α-globin gene cluster spans ≈25.5 kbp (*C*). From electron micrographs of λCαG2 (see Fig. 4), the precise localization, positions of the intervening sequences, and direction of transcription of the adult α-globin genes were deduced (*D*). Also shown is the probable position of the embryonic globin complementary locus, U. ⇓, *Eco*RI linker; ↓, *Eco*RI; ↑, *Bam*HI; ▽, *Hin*dIII; △, *Kpn* I.

reported by Salser *et al.* (16). For the present, we have designated this homology region U (Fig. 2*D*).

Electron Microscopic Analysis of λCαG2. We wished to compare the structure of the α^A and α^D chicken globin genes to the mouse α-globin gene described by Leder *et al.* (19). We used the technique of electron microscopic visualization of R loops (14, 15) in which mRNA·DNA hybrids are observed on the intact recombinant phage DNA molecule. Adult globin mRNA was hybridized to trioxalen-crosslinked λCαG2 DNA (the trioxalen treatment was necessary to prevent complete DNA strand separation). An example of the resultant R-loop structure is shown in Fig. 4*A*. Each phage DNA molecule observed contained two separate R-loop structures (about 80% labeling for each R loop). The two R-loop regions mapped to the regions of globin cDNA homology shown in Fig. 2*A*. The structures of the two R loops were somewhat variable, but, in general, there was no observable difference between the R-loop region proximal to the nearest end of the phage DNA molecule (α^D, see above) and the distal R-loop structure (α^A). The distance from the nearest end of the phage DNA to the α^D R loop is 11.5 ± 0.5 kbp (SD, n = 19) and the distance to the α^A R loop is 14.6 ± 0.6 kbp. The two R-loop structures are separated by 2.3 ± 0.2 kbp of DNA (end-to-end distances given in all cases). These measurements would indicate that the α^A gene depicted in Fig. 2*D* would lie in the most distal position (with respect to the α^D gene) of the *Hin*dIII/*Bam*HI restriction fragment defining α^A (Fig. 2*A*).

The variability observed in the R-loop structures is due to the presence of two small intervening sequences (IVS) which interrupt the coding regions of each of the α-globin genes. All four IVS regions on λCαG2 (two in each gene) are small enough that they are observed either as a small loop of renatured IVS DNA

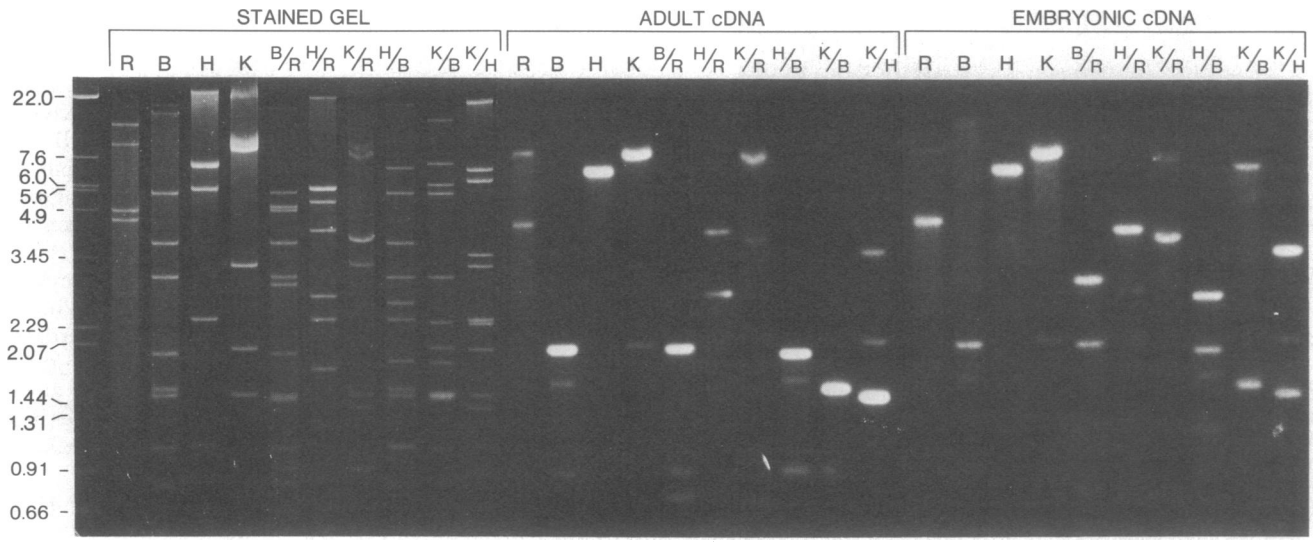

FIG. 3. Hybridization of stage-specific chicken globin cDNA to λCαG5. λCαG5 DNA was cleaved with *Eco*RI (R), *Bam*HI (B), *Hin*dIII (H), and *Kpn* I (K) and with all paired combinations of these. The DNA was then electrophoresed, stained, photographed ("stained gel"), and blotted to nitrocellulose (5). The immobilized DNA on the nitrocellulose was then hybridized to adult and to embryonic chicken globin cDNA, successively (ref. 5; and see legend to Fig. 1). Size standards, shown in kbp on the left, are λ/*Eco*RI (partial) (11, 12) and pBR322 cleaved with *Eco*RI and *Pvu* II (2.29 and 2.07 kbp), with *Taq* I (1.44 and 1.31 kbp), and with *Alu* I (0.91 kbp) (13). Lanes designated "adult" and "embryonic" represent autoradiograms of the filter hybridized to adult globin or 5-day embryonic globin cDNA, respectively. Exonuclease contamination during digestion was apparently the cause of some bands in the gel being present at less than stoichiometric amounts. This can be seen in the stained photograph as a lane-specific haze or background. Globin sequences were present in excess on the nitrocellulose; thus the relative abundance of the different genes present in the two cDNA populations is proportional to the intensity of the bands.

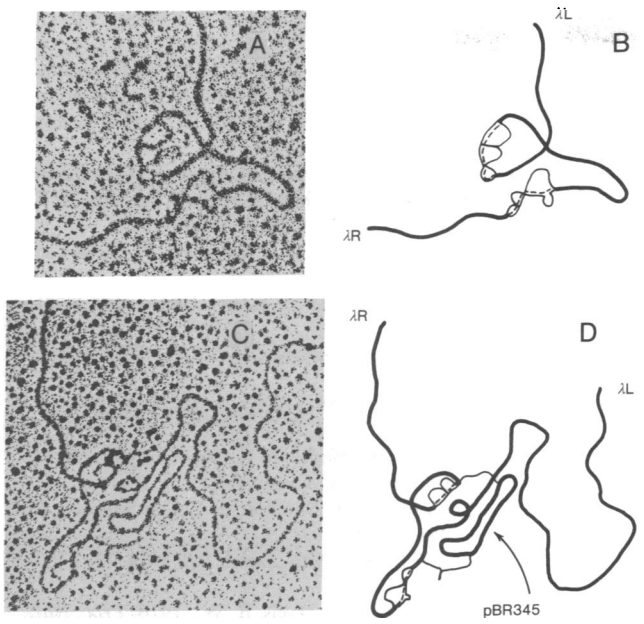

FIG. 4. Position of intervening sequences in and orientation of adult chicken globin genes in λCαG2. DNA was crosslinked with trioxalen and hybridized to adult globin mRNA as described (5, 6, 15). R loops were fixed with glyoxal, spread, shadowed, and examined with a Phillips 300 electron microscope as described (15). (*A*) Typical example of α-globin R-loop structures on λCαG2 (approximately 20% of the whole recombinant DNA molecule); (*B*) an interpretive drawing (dashed lines represent mRNA in the mRNA·DNA duplex). (*C*) R loops were prepared as described above; then circular pBR345 plasmid DNA [on which single-stranded "tails" of poly(BrdU) had been synthesized] was added to the mixture, which was incubated at room temperature and spread as described (5, 15). A molecule of λCαG2 DNA in which both R-loop structures have been labeled at their 3′ termini by a single plasmid DNA molecule is shown. (*D*) An interpretive drawing of the micrograph in *C*.

or as a knob of single-stranded DNA extending from the RNA·DNA hybrid region of an R loop. The latter structure presumably results from IVS DNA that has not renatured with its complementary region in the displaced DNA strand. Such structures commonly occur in R loops with small IVS regions (6, 19, 20) probably as a result of kinetic, thermodynamic, or topological restraints on IVS DNA loop renaturation. All four IVS loops can be estimated from the electron micrographs to be 100–150 base pairs (bp) long; however, this measurement is rather inaccurate due to the variability of the R-loop structures and probably represents a slight underestimate. These α-globin gene IVS regions are more consistently visible in the micrographs than the 100-bp small IVS of the adult chicken β-globin gene (5, 6), but this may be a result of the G+C content of the DNA rather than the length of these particular IVS regions.

The location of the corresponding IVS regions in the α^A and α^D R loops are indistinguishable by electron microscopy and appear to be located similarly to those of the mouse α-globin gene (ref. 19 and see *Discussion*). For both genes the coding region blocks are about 160, 180, and 180 bp, respectively, reading in the 5′→3′ transcriptional direction (see below). (These again represent lower estimates. If the IVS locations are equivalent in mouse and chicken α-globin genes, the central coding region would be 204 bp long.)

Previous studies of linked β-globin genes suggested that all genes of the β cluster are transcribed in the same direction (5, 21, 22). The transcriptional direction of the α^A and α^D chicken globin genes was determined by labeling the free 3′-poly(A) portion of the R loops of λCαG2 with a plasmid DNA (pBR345) to which had been added single-stranded "tails" of poly(BrdU) (15, 23). The position of the circular plasmid DNAs thereby orient the direction of transcription of the mRNA in the loop and, thus, the corresponding gene. Such a micrograph of λCαG2 is shown in Fig. 4*C*. Poly(BrdU) tails from a single plasmid DNA can be seen to extend to each of the α-globin R loops. Examination of the orientation of the R loops in Fig. 4*C* shows that these two genes are indeed transcribed in the same direction. A lower magnification of this molecule and micrographs of several other labeled λCαG2 R loops (not shown) demonstrate that the two genes are transcribed in the order 5′-α^D-α^A-3′.

DISCUSSION

In this paper we describe the isolation and initial characterization of recombinant chromosomal chicken DNA fragments that contain the adult chicken α-globin genes, α^A and α^D. The chromosomal *Eco*RI restriction fragment containing the major adult α-globin sequence, α^A, is present intact in one recombinant (λCαG2), whereas the chromosomal *Eco*RI fragment containing the α^D gene, the minor adult α-globin gene in chickens, is represented completely in λCαG5. The sizes of these isolated *Eco*RI fragments agree with our initial results using restriction digests of total chromosomal DNA (9). Thus we have isolated two closely linked α-globin genes from chromosomal DNA in a manner analogous to the isolation of linked β-globin genes in chickens (5), rabbits (4, 21), and humans (24).

Recently, Orkin (25) has described, by Southern blotting experiments, the chromosomal linkage of the duplicated adult human α-globin genes. These genes have been isolated and are being characterized (J. Lauer and T. Maniatis, personal communication). Unlike the human α-globin gene linkage group, which contains two genes each coding for an identical α-globin polypeptide (26), the adult α-globin genes of chickens are widely divergent and are expressed in approximately a 3:1 (α^A:α^D) ratio *in vivo* (1). This situation may therefore be more analogous to the relative expression of mouse β-major and β-minor genes (expressed in approximately a 4:1 ratio) than to mammalian α-globin genes. The possibility of course exists that the duplicated adult α-globin genes of humans, although coding for identical polypeptide gene products, are not expressed with equal efficiency, perhaps because of changes in DNA sequences adjacent or internal to their coding regions.

This work, when considered with previous studies of the mouse α-globin gene (19), demonstrates that the structure of α-type globin genes is highly conserved evolutionarily just as are the β-type gene structures (5, 20, 21, 24). That the locations of globin gene intervening sequences have been conserved from before the initial α-β divergence was suggested by Leder *et al.* (19), and this prediction is supported by the work described in this paper. The approximate sizes of the IVS regions are also conserved within (but not between) the α- and β-globin gene families. This is true even for the chicken α^A and α^D globin genes, which are even more widely divergent than are the α^A and the rabbit α-globin genes (ref. 9 and Fig. 1). This suggests that IVS sizes were set during the early divergence of α-like and β-like globins. The size of the IVS regions in the chromosomal genes determines the length of IVS RNA that must be processed out of the initially transcribed precursor RNA. The evolutionary stability of IVS size may reflect an interaction between precursor RNA structure and functionally important (and therefore highly conserved) processing or transport mechanisms (or both) in the cell (27). It is therefore interesting that the 5′-IVS of α-type globin genes is similar in size to the corresponding IVS

of β-type globin genes (about 100–150 bp) whereas α-type globins have a small 3′-IVS (100–200 bp) compared to β-type genes (500–900 bp). Thus, the 5′-IVS could be used as a general globin transcript regulatory signal, and the 3′-IVS could be used to differentiate α-type and β-type globin expression.

One further point related to chicken α-globin sequence arrangement emerges from this study of the adult α-globin genes. The homology region designated U (Fig. 2*D*) contains DNA sequence homology only to embryonic globin mRNA. Based on previously reported data (9), we would assume this to be the structural gene for globin π or π', the most abundant embryonic α-type globin (6). If this is indeed the π or π' structural gene region, an important question arises concerning the embryonic α-globin genes. Are the closely related but structurally distinct π and π' globin chains the result of simple allelic variation or are the two polypeptides encoded by two separate genes? Bearing on this question, we have recently isolated a third recombinant in the α-globin gene region. This recombinant contains sequence overlap to region U of $\lambda C\alpha G5$ and extends away from the α^A and α^D genes (in a 5′ transcriptional sense to these genes). In the third genomic segment uniquely defined in this recombinant, no new adult or embryonic globin complementary sequences have been found. From this observation, any additional globin genes at the α-globin locus must be a minimum of 15 kbp from sequence U (distal to α^A and α^D). Thus, if π and π' are distinct genes, they must both be encoded within $\lambda C\alpha G5$ or they must be separated by >15 kbp in the chromosome.

Some of this work was done at the California Institute of Technology in the laboratory of Norman Davidson, whose generous support and encouragement is gratefully acknowledged. We also thank Tom Maniatis for advice and gifts of rabbit globin probes; Pierre Chambon and Winston Salser for gifts of globin cDNA plasmids; and David Kaback for the 3′-label used in the electron microscopy. We thank M. Dolan and D. Zimmerman for technical assistance. J.B.D. was supported by a postdoctoral fellowship from National Institutes of Health (GM 06921); J.D.E. acknowledges postdoctoral fellowship support from the Helen Hay Whitney Foundation. Latter stages of this work were funded by Grants 78-77 from the American Cancer Society, Illinois Division, the Northwestern University Research Committee, and HL24415 and CA15145 from National Institutes of Health (J.D.E.).

1. Brown, J. L. & Ingram, V. M. (1974) *J. Biol. Chem.* **249,** 3960–3972.
2. Romanoff, A. L. (1960) *The Avian Embryo, Structural and Functional Development* (MacMillan, New York).
3. Bruns, G. A. P. & Ingram, V. M. (1973) *Dev. Biol.* **30,** 455–459.
4. Maniatis, T., Hardison, R. C., Lacy, E., Lauer, J., O'Connell, C., Quon, D., Sim, G. K. & Efstratiadis, A. (1978) *Cell* **15,** 687–701.
5. Dodgson, J. B., Strommer, J. & Engel, J. D. (1979) *Cell* **17,** 879–887.
6. Dodgson, J. B., Strommer, J. & Engel, J. D. (1979) in *Eucaryotic Gene Regulation*, Eighth Annual ICN–UCLA Symposium on Molecular and Cellular Biology, eds. Maniatis, T., Axel, R. & Fox, C. F. (Academic, New York).
7. Hughes, S. H., Stubblefield, E., Payvar, F., Engel, J. D., Dodgson, J. B., Spector, D., Cordell, B., Schimke, R. T. & Varmus, H. E. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1348–1352.
8. Maniatis, T., Sim, G. K., Efstratiadis, A. & Kafatos, F. (1976) *Cell* **8,** 163–182.
9. Engel, J. D. & Dodgson, J. B. (1978) *J. Biol. Chem.* **253,** 8239–8246.
10. Legerski, R. J., Hodnett, J. L. & Gray, H. B., Jr. (1978) *Nucleic Acids Res.* **5,** 1445–1464.
11. Thomas, M. O. & Davis, R. W. (1975) *J. Mol. Biol.* **91,** 315–328.
12. Blattner, F. R., Williams, B., Blechl, A. E., Denniston-Thompson, K., Faber, H. E., Furlong, L. A., Grunwald, D. G., Kiefer, D. O., Moore, D. D., Schumm, J. W., Sheldon, E. L. and Smithies, O. (1977) *Science* **196,** 161–169.
13. Suttcliffe, J. G. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 3737–3741.
14. Thomas, J. O., White, R. L. & Davis, R. W. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2294–2298.
15. Kaback, D., Angerer, L. A. & Davidson, N. (1979) *Nucleic Acids Res.* **6,** 2499–2517.
16. Salser, W. A., Cummings, I., Liu, A., Strommer, J., Padayatty, J. & Clarke, P. (1979) in *Cellular and Molecular Regulation of Hemoglobin Switching*, eds. Stamatoyannopoulos, G. & Nienhuis, A. (Grune and Stratton, New York), pp. 621–643.
17. Flamm, U., Best, J. S. and Braunitzer, G. (1971) *Hoppe-Seyler's Z. Physiol. Chem.* **352,** 885–895.
18. Matsuda, G., Takei, H., Wu, K. C. & Shiozawa, T. (1971) *Int. J. Prot. Res.* **3,** 173.
19. Leder, A., Miller, H. I., Hamer, D. H., Seidman, J. G., Norman, B., Sullivan, M. & Leder, P. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 6187–6191.
20. Tilghman, S. M., Tiemeier, D. C., Seidman, J. G., Peterlin, B. M., Sullivan, M., Maizel, J. V. and Leder, P. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 725–729.
21. Lacy, E., Hardison, R. C., Quon, D. & Maniatis, T. (1979) *Cell* **18,** 1273–1284.
22. Fritch, E. F., Lawn, R. M. & Maniatis, T. (1979) *Nature (London)* **279,** 598–603.
23. Bender, W. and Davidson, N. (1976) *Cell* **7,** 595–607.
24. Lawn, R. M., Fritsch, E. F., Parker, R. C., Blake, G. and Maniatis, T. (1978) *Cell* **15,** 1157–1174.
25. Orkin, S. H. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5950–5954.
26. Old, J. M., Clegg, J. B., Weatherall, D. J. & Booth, P. B. (1978) *Nature (London)* **273,** 319–320.
27. Gruss, P., Lai, G.-J., Dhar, R. & Khoury, G. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4317–4321.