

# Isolation and Sequencing of Two $\alpha$ -Globin Genes $\alpha^A$ and $\alpha^D$ in Pigeon and Evidence for Embryo-Specific Expression of the $\alpha^D$ -Globin Gene<sup>1</sup>

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**By screening a pigeon genomic DNA library, we isolated a recombinant phage clone containing the  $\alpha^A$ -globin gene. The DNA sequence of the approximately 6kbp-long insert fragment of the phage clone was determined. The sequence suggested the existence of pigeon  $\alpha^D$ -globin gene located 3.1 kbp upstream from the  $\alpha^A$ -globin gene. The expression of the  $\alpha^D$ -globin in late embryo was also shown by the N-terminal amino-acid sequence of the intact globin chain. These results show that two adult  $\alpha$ -globin genes,  $\alpha^A$  and  $\alpha^D$ , exist in the pigeon genome, and the  $\alpha^D$ -globin is expressed at the late embryo stage. The stage-specific expression suggests the existence of regulatory elements and factors interacting to inhibit transcription at the adult stage.**

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The globin gene family has been useful for studying stage- and tissue-specific gene expression in eukaryotes. Particularly, the chicken globin families are well characterized as developmentally regulated genes. The  $\alpha$ -globin gene family consists of three closely linked genes, two ( $\alpha^A$ - and  $\alpha^D$ -globin, adult type  $\alpha$ -globin genes) of which are expressed in both primitive (present from 2 to 5 days of embryonic life) and definitive (present from 6 days embryo through adult life) cell lineages and one ( $\pi$ -globin) of which is expressed in a primitive lineage (1). The three genes are arranged in order of their expression during development, 5' -  $\pi$  -  $\alpha^D$  -  $\alpha^A$  - 3'. The complete nucleotide sequences of the  $\alpha^A$ - and  $\alpha^D$ -genes, which are very different in sequence,

were reported by Dodgson and Engel (2). The  $\alpha^A$ - and  $\alpha^D$ -globins were detected at a ratio of approximately 3:1 in definitive lineage (late embryo and adult stage) and at a ratio of approximately 2:1 in primitive lineage (early embryonic stage) (3). This finding indicates that the proportion of  $\alpha^A$ - and  $\alpha^D$ -globins vary during development. Most avian species generally have two adult type  $\alpha$ -globin genes ( $\alpha^A$  and  $\alpha^D$ ), as shown in the chicken(2). Although DNA sequences of these genes and identification and characterization of *cis*-regulatory elements near the members of the gene family were studied in several species (2,4-6), the mechanism of their coordinate expression during development and why avian species have two very different adult  $\alpha$ -globin genes remain unclear.

In contrast to most avian species having two adult  $\alpha$ -globins ( $\alpha^A$  and  $\alpha^D$ ) such as chicken (7), turkey (8), goose (9), and goshawk (10), several avian species such as pigeon (11), parakeet (12), penguin (13), and Blue-and-Yellow Macaw (14) do not have  $\alpha^D$ -globin at the adult stage. Therefore no information is available on the  $\alpha^D$ -globin gene in these species. Providing some information on the  $\alpha^D$ -globin gene in these species would be a valuable contribution to the study of coordinate gene expression during development and the estimate of molecular evolution of  $\alpha$ -globin in avian. In the present experiment, we examined whether or not the  $\alpha^D$ -globin gene existed upstream from the  $\alpha^A$ -globin gene in pigeon, which was described with the complete nucleotide sequence of  $\alpha^A$ -globin cDNA (15). We isolated a recombinant phage clone containing pigeon  $\alpha^D$ -globin gene located 3.1 kbp upstream from the  $\alpha^A$ -globin gene by screening a pigeon genomic DNA library, and the DNA sequence of the approximately 6 kbp-long insert fragment including the two adult  $\alpha$ -globin genes,  $\alpha^A$  and  $\alpha^D$ , was determined. The deduced amino-acid sequence of  $\alpha^D$ -globin was used for the construction of phylogenetic tree to estimate the evolutionary distance between pigeon and other avian species. The hemoglo-

<sup>1</sup> The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases under Accession No. AB001981.

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bin components in the late embryo stage were also studied. The results show that the  $\alpha^D$ -globin gene exists in the pigeon genome and is expressed only in the late embryo but not in the adult stage.

insert was sequenced after subcloning into pUC19 vector. DNA sequencing was performed using a Thermo Sequenase Sequencing Kit (Amersham) with an automated DNA sequencer (Model SQ5500, HITACHI).

MATERIALS AND METHODS

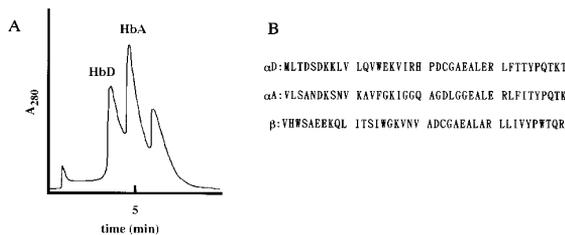
**Cloning and DNA sequencing.** A pigeon genomic DNA library (lambda GEM-12) was obtained from Prof. Nelson Horseman (Department of Physiology and Biophysics, University of Cincinnati, Ohio, USA) and screened with pigeon  $\alpha^A$ -globin cDNA probe (15) using ECL Direct nucleic-acid labelling and detection system (Amersham). A positive lambda phage clone containing a 15kbp genomic DNA insert was isolated and denoted as  $\lambda P\alpha$ -1. About 6kbp long

**Separation of hemoglobin components and amino acid sequencing.** Pigeon erythrocytes were obtained from 7 to 16 day old embryos. The hemoglobin components were separated by ion-exchange chromatography on a CM-Men Sep cartridge (Millphore Co. USA). The column was equilibrated with 20mM phosphate buffer (pH 6.3). The hemoglobin components were eluted with a linear gradient of 0-0.1M NaCl over 7min at a flow rate of 1.5ml/min. The absorbance was monitored at 280nm. The  $\alpha$ - and  $\beta$ -globins from hemoglobin were separated by reversed-phase HPLC using a column containing  $\mu$ -Bondasphere C4.

The first 40 residues or so from the N-terminus of intact globins

CGATCAGGTTACATTTACTGCCATGCTCTCAGAGGAATCTGACACGAAAAGGTGGGCACAAATCTTAAAGCACACTGTAGTGTACAACTGAGCTGGCACTACAAGCTGTTCCTCATCCCGTTTACAAAAT 140
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AACACACACTGGGGTGGGAAGAAGAAAGAACAGCCGCTGACAAGCATCAGAGGATGGAATTTGGGAAGACTATGAGCCTGAAAAGGAGATTTCCCCACTCAGGCTCTCCAGGATGCTGGGGAGATGCTGTTTCC 560
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M L T D S T D
CAAGAAGCTGGTCTGACGGTGTGGGAGAAGGTGATCCGCCACCCAGACTGTGAGCCGAGGCCCTGGAGAGGTGCGGGCTGAGCTGGGGAAACCATGGGCAAGGGGGCGACTGGGTGGAGCCCTACAGGGCTGCTG 1260
K K L V L Q V W E K V I R H P D C G A E A L E R
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L F T T Y P Q T K T Y F P H F D L H H G S D Q V R N H G K K V L
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A A F D K F L S A V C T V L A E K Y R
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GCTGTGGAGCT 5981

FIG. 1. Nucleotide and deduced amino-acid sequence of the  $\alpha$ -globin gene. The amino acid sequences are shown in one-letter code.



**FIG. 2.** Separation of hemoglobin components of pigeon embryonic blood by ion-exchange chromatography (A). N-terminal amino acid sequences of pigeon globin chains in embryonic blood (B). For details see "Materials and methods".  $\alpha^D$ :  $\alpha^D$ -globin chain,  $\alpha^A$ :  $\alpha^A$ -globin chain,  $\beta$ :  $\beta$ -globin chain.

were automatically sequenced. The amino-acid sequences were determined using a gas phase automatic sequence analyzer (Model PSQ-1, Shimadzu Co., Kyoto) equipped with an online PTH analyzer (Model, PTH-1, Shimadzu Co.).

**Analysis of  $\alpha$ -globin chains.** The  $\alpha$ -globin amino-acid sequence data of the other avian and Western painted turtle were downloaded from the SWISS-PROT database. These sequence data and deduced amino acid sequence of pigeon  $\alpha^A$ - and  $\alpha^D$ -globin gene were used for the neighbor-joining analysis. The analysis was performed using programs in the PHYLIP package (version 3.5c) from Felsenstein (16,17).

## RESULTS

### DNA Sequence of the Two Pigeon $\alpha$ -Globin Genes

The phage clone,  $\lambda P\alpha$ -1, containing approximately a 15 kbp-long insert fragment including the  $\alpha^A$ -globin gene, was isolated by screening the pigeon genomic DNA library and subcloned in plasmid pUC19. The homologous region (pigeon  $\alpha^D$ -like) of the chicken  $\alpha^D$ -globin gene located 3.1kbp upstream from the pigeon  $\alpha^A$ -globin gene. The nucleotide sequence of the 6kbp long region contained the  $\alpha^D$ -like and  $\alpha^A$ -globin gene and the deduced amino-acid sequences are shown in Fig.1. These sequences show that the pigeon genome contains the  $\alpha^D$ -globin gene encoding a 140 amino acid polypeptide. The sequence of the  $\alpha^A$ -globin gene largely matches with that derived from the cDNA clone described by Eguchi et al. (15), although differences occur at positions 15 and 130 of the  $\alpha^A$ -globin chain between the sequence from genomic clone and that from cDNA clone.

### Detection of $\alpha^D$ -Globin Chain at Embryo Stage

We surveyed hemoglobin components in pigeon erythrocytes of late embryo stage (7 to 16 day old embryos). The erythrocytes of the pigeon late embryo contained two hemoglobin components. These two components, hemoglobin A (HbA) and hemoglobin D (HbD), were separated by ion-exchange chromatography on a CM-Men Sep cartridge column. The elution profiles of the HbA and HbD are shown in Fig.2A. The  $\alpha$ - and  $\beta$ -globin chains from hemoglobin components of embryo

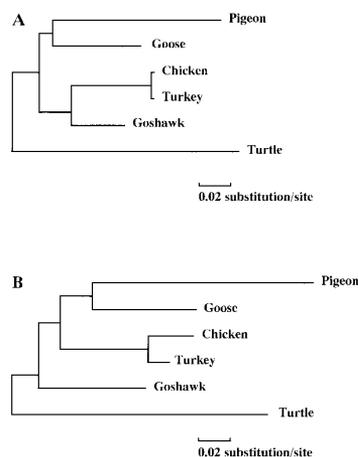
stage were separated by reversed-phase HPLC using a  $\mu$ -Bondasphere C4 column (the elution profiles of the  $\alpha$ - and  $\beta$ -globin chains are not shown). The first 40 residues or so from the N-terminus of intact globins were sequenced (Fig. 2B). The N-terminal amino acid sequence of the  $\alpha$ -globin chain was identical with the deduced sequence of the  $\alpha^D$ -globin gene coding region in pigeon genomic DNA clone ( $\lambda P\alpha$ -1).

### Evolutionary Distance of $\alpha$ -Globin Chains

We constructed phylogenetic trees from  $\alpha$ -globin amino acid sequence data by neighbor-joining (NJ) (Fig.3A and B) using Reptilia (Western Painted turtle) as an outgroup. The evolutionary distance between pigeon and chicken is 0.18 substitution per site for the  $\alpha^A$ -globin chain and 0.23 for the  $\alpha^D$ -globin chain. These values indicate that the amino acid substitution rate is higher in  $\alpha^D$ -globin chain than in  $\alpha^A$ .

## DISCUSSION

The recombinant phage clone,  $\lambda P\alpha$ -1, containing two pigeon  $\alpha$ -globin genes,  $\alpha^A$  and  $\alpha^D$ , was isolated by screening the pigeon genomic DNA library with a pigeon  $\alpha^A$ -globin cDNA probe. The nucleotide sequence of a 6 kbp long, including the two  $\alpha$ -globin genes, were determined from this phage clone (Fig.1). Additionally, two types of hemoglobin components, HbA and HbD, were detected in pigeon late embryonic blood by ion-exchange chromatography. The N-terminal amino acid sequence of the globin chain shows that the  $\alpha^D$ -globin is expressed at the late embryo stage (Fig.2). Amino acid sequence differences occurred at positions 15 and 130 of the  $\alpha^A$ -globin chain between the sequence from genomic clone and that previously reported from cDNA clone (15). These differences may be due to sequencing



**FIG. 3.** Phylogenetic trees inferred by neighbor-joining for the  $\alpha^A$ -globin chain (A) and  $\alpha^D$ -globin chain (B). Western painted turtle (Reptilia) was used as an outgroup.

errors in the cDNA, because at position 15, the result from the genomic DNA is supported from the amino acid sequence data (Fig.2B), and there is a Cys at position 130 in most avian species.

The  $\alpha$ -globin gene family of avian species consists of three closely linked genes,  $\pi$ ,  $\alpha^D$  and  $\alpha^A$ . In contrast to primitive cells' specific  $\pi$  gene, the expression of  $\alpha^A$  and  $\alpha^D$  is seen in both primitive and definitive cells. Knezetic and Felsenfeld (18) proposed that the concentration-dependent action of three factors, an NF1 family member, a Y-box factor, and an Sp1-like factor, is responsible for the stage-specific expression of the  $\pi$  gene. However, the mechanism of the coordinate gene expression of the adult globin genes,  $\alpha^A$  and  $\alpha^D$ , remains unknown. A silencer and an enhancer elements located at the 3'-side of the chicken  $\alpha$ -globin gene domain were identified (6). We consider the possibility that the stage-specific expression of the  $\alpha^D$ -globin gene in pigeon is controlled by a different mechanism than the embryonic globin gene,  $\pi$ . It is tempting to speculate that the interaction of these regulatory elements and promoter region reduces the activity of the  $\alpha^D$ -globin promoter at the adult stage.

Hiebl et al. (9, 19) have considered two possibilities: one, that the  $\alpha^D$ -gene is a biological reserve to situationally enlarge the normal hemoglobin function in avian species which need hemoglobins to adjust quickly to different environments, such as near sea-level to high altitudes; and two, that it is an intermediate between a functional gene, reduced in its expression, and a pseudogene evidenced by its low expression rate (approximately 10-25%) in three species of goose. However, the  $\alpha^D$ -gene has been expressed in adult chickens and turkeys having inferior ability for flying. A pigeon adult  $\alpha$ -globin gene,  $\alpha^A$  and  $\alpha^D$ , were expressed at the late embryo stage (Fig.2). Godovac-Zimmermann and Braunitzer (14) suggested that avian  $\alpha^D$ -globin chains possibly, have no functional importance in the adult, because avian  $\alpha^D$ -globin chains have been reported to show a much higher evolutionary rate than  $\alpha^A$ -globin chains (20). This is in accordance with the neutral mutation-random drift hypothesis (21). The  $\alpha^D$ -globin chain of pigeon showed a much higher evolutionary rate than  $\alpha^A$ -globin chain (Fig.3). Thus, the  $\alpha^D$ -globin chain may not be needed for adult life, although the pigeon  $\alpha^D$ -globin is expressed in the late embryo stage. This study will serve as a basis for studying the coordinate expression and the molecular evolution of the  $\alpha$ -globin gene family in avian.

## ACKNOWLEDGMENTS

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## REFERENCES

1. Bruns, G. A., and Ingram, V. M. (1973) *Phil. Trans. R. Soc. Ser. B* **266**, 225-305.
2. Dodgson, J. B., and Engel, J. D. (1983) *J. Biol. Chem.* **258**, 4623-4629.
3. Brown, J. L., and Ingram, V. M. (1974) *J. Biol. Chem.* **249**, 3960-3972.
4. Kemper, B., Jackson, P. D., and Felsenfeld, G. (1987) *Mol. Cell. Biol.* **7**, 2059-2069.
5. Knezetic, J. A., and Felsenfeld, G. (1989) *Mol. Cell. Biol.* **9**, 893-901.
6. Recillas Targa, F., De moura Gallo, C. V., Huesca, M., Scherrer, K., and Marcaud, L. (1993) *Gene* **129**, 229-237.
7. Takei, H., Ota, Y., Wu, K. C., Kiyohara, T., and Matsuda, G. (1975) *J. Biochem.* **77**, 1345-1347.
8. Eguchi, Y., Ikehara, T., Kayo, S., Eguchi, T., and Takei, H. (1995) *Biol. Chem. Hoppe-Seyler* **376**, 437-440.
9. Hiebl, I., Braunitzer, G., and Schneeganss, D. (1987) *Biol. Chem. Hoppe-Seyler* **368**, 1559-1569.
10. Hiebl, I., Kusters, J., and Braunitzer, G. (1987) *Biol. Chem. Hoppe-Seyler* **368**, 333-342.
11. Sultana, C. A., and Zaidi, Z. H. (1989) *J. Prot. Chem.* **8**, 629-646.
12. Islam, A., Beg, O. U., Persson, B., Zaidi, Z. H., and Joernvall, H. (1988) *J. Prot. Chem.* **7**, 561-569.
13. Huber, K., Braunitzer, G., Schneeganss, D., Kusters, J., and Grimm, F. (1988) *Biol. Chem. Hoppe-Seyler* **369**, 513-519.
14. Godovac-Zimmermann, J., and Braunitzer, G. (1985) *Biol. Chem. Hoppe-Seyler* **366**, 503-508.
15. Eguchi, Y., Nakashima, Y., Oshiro, M., and Takei, H. (1990) *Nucleic Acids Res.* **18**, 7135.
16. Felsenstein, J. (1989) PHYLIP-Phylogeny Inference Package (Version 3.2). *Cladistics* **5**, 164-166.
17. Felsenstein, J. (1993) PHYLIP (Phylogeny Inference Package) Version 3.5c. Department of Genetics, University of Washington, Seattle. [distributed by the author]
18. Knezetic, J. A., and Felsenfeld, G. (1993) *Mol. Cell. Biol.* **13**, 4632-4639.
19. Hiebl, I., Schneeganss, D., and Braunitzer, G. (1986) *Biol. Chem. Hoppe-Seyler* **367**, 591-599.
20. Oberthur, W., Godovac-Zimmermann, J., Braunitzer, G., and Wiesner, H. (1982) *Hoppe-Seyler's Z. Physiol. Chem.* **363**, 777-787.
21. Kimura, M. (1979) *Sci. Ame.* **241**, 94-104.