CHICKEN GLOBIN mRNA AND GENES

A thesis submitted for
the Degree of
Doctor of Philosophy
in the
University of Adelaide
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April 1980

awarded May 1981

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SUMMARY

The work in this thesis concerns the characterization of globin mRNA from avian erythroid cells. The primary structure of the major mRNA species was deduced by sequence analysis of recombinant DNA clones containing cDNA inserts derived from purified polysomal chicken globin mRNA.

1. Anaemia was induced in 12 week old chickens by the injection of phenylhydrazine. Polysomes were isolated from the blood and the 9S RNA purified. Double-stranded DNA copies of the mRNA were synthesized by sequential reverse transcriptase reactions, with oligo-(dT) priming of the initial reaction. Double and single-stranded cDNA were subjected to cleavage with restriction endonucleases to characterize the sequences represented.
2. Full length double-stranded cDNA was inserted into the plasmid BR322 by the use of synthetic linker DNA containing the *Hin*dIII recognition sequence. Inserted DNA from the individual recombinants was purified from the plasmid DNA and subjected to sequence analysis by the chemical degradation method of Maxam and Gilbert.
3. The complete sequence of the longest inserts representing the two major mRNA transcripts was deduced.

This work produced the following conclusions:-

a) there are two major globin mRNA species present in the erythroid cells of phenylhydrazine induced anaemic chickens. The β coding species codes for a protein which is identical to that in non-anaemic adult blood. The α coding species codes

for a protein which is not like either of the α chains present in non-anaemic adult blood, and therefore must be induced by phenylhydrazine treatment,

b) the induced α coding mRNA codes for a protein which differs by 22 amino acids from that of α_A and by 61 amino acids from that of α_D, these two alpha chains being the normal components of adult chicken globin,

c) sequence analysis of several recombinants reveals that errors are made during the cloning process due to the necessity of the reverse transcriptase enzyme to use a loop structure of the first DNA strand as the primer for second strand synthesis. These "errors" are confined to the 5' end (with respect to the mRNA) of the inserts,

d) comparison of the 3' untranslated regions with mammalian globin mRNA sequences reveals homology which is consistent with chicken globin genes being relatively primitive and having origins close to the separation of globin into α and β chain types on the genealogical tree.