ERRATA

p 46, line 21 should read: "5 minutes. The nuclei were collected by centrifugation."

p 47, lines 6 and 8. The pancreatic ribonuclease and pronase were dissolved in standard saline citrate at 2 mg/ml prior to adding to the DNA.

Fig. 3.2 legend should read: "A. ^{14}C-globin"
"B. ^{14}C-H5 plus ^{14}C-globin"
"C. ^{14}C-H5"

Fig. 6.5. The concentration of acrylamide in these gels is 4%.

CHICKEN HISTONE H5 mRNA

AND ITS GENES

A thesis submitted for

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by

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SUMMARY

1. The work described in this thesis forms part of an investigation of eukaryotic gene control. The system studied was the avian erythroid cell series since it is possible to isolate pure populations of the various cell types which have well-defined biochemical activities. These cells contain an unusual tissue-specific histone H5, which may be involved in the progressive repression of transcription observed as these cells differentiate. Although the gene controlling function of this histone must be at a very gross level, this represents a unique opportunity to investigate one facet of gene control. Probably the most sensitive technique is to assay for specific messenger RNA and gene sequences by hybridisation to an appropriate probe. The aim of this thesis was to prepare such a probe from H5 mRNA and to use it to calculate the reiteration frequency of the H5 gene in the chicken genome.

2. The cells employed were chicken reticulocytes since the only histone made in these cells is H5. Experiments were conducted which demonstrated that H5 mRNA is probably a minor species compared to globin mRNA in these cells. Furthermore, calculations indicate that the two mRNAs are probably of similar molecular weight which may complicate the isolation of H5 mRNA. As a result globin mRNA was first purified and characterised. Properties which may have proved useful in the separation of this mRNA from H5 mRNA are discussed. The globin mRNA was used to optimise techniques for the *in vitro* translation and identification of chicken mRNAs. This was considered necessary as mRNAs

from different sources vary in the conditions required for optimal translation and it was reasoned that mRNAs from the same cell would have similar optima.

3. Total polysomal RNA was fractionated on the basis of size and poly A content. Although large amounts of globin mRNA were present, H5 mRNA could only be detected in the non-poly A containing RNA. Even in this fraction however, there was still a large excess of globin mRNA which was difficult to remove due to the demonstrated similarity of their molecular weights.

4. Since it had proved impossible to isolate the H5 mRNA by conventional techniques, immunological methods of isolating the polysomes producing H5 were investigated. Using immunoadsorbents, mRNA was prepared in small amounts which programmed the synthesis *in vitro* of more than 70% H5. The yield and specificity were improved by modifying the procedure to indirect immunoprecipitation followed by oligo(dT)-cellulose chromatography. The resulting mRNA programmes the synthesis *in vitro* of more than 90% H5. The chemical purity of the mRNA is discussed.

5. The immunologically prepared H5 mRNA was not copied into cDNA by RNA-dependent DNA-polymerase. Since this was probably due to the lack of a 3' poly A tract on the mRNA, an enzyme was purified and characterised which would add such a tract. The enzymically modified mRNA could then be copied into cDNA of high specific activity.

6. The H5 cDNA was characterised in terms of size and fidelity of copying. By hybridisation analysis it was dem-

onstrated that the amount of contaminating rRNA and globin mRNA complementary sequences present in the cDNA was insignificant. The complexity of the cDNA was shown to be of the same size as the H5 mRNA and will back hybridise to this mRNA to greater than 75%. These results are discussed to demonstrate that the cDNA is a faithful copy of H5 mRNA. The possible uses of the resulting probe are also discussed.

7. The H5 cDNA was employed to quantify the number of H5 genes in the chicken genome. The significance of this result is discussed in terms of the known reiteration and organisation of histone genes in other species, and the possible role of H5 as a gene control agent.

STATEMENT

This thesis contains no material which has been accepted for the award of any other Degree or Diploma of any University, and to the best of my knowledge and belief contains no material previously published or written by any other person, except when due reference is made in the text.

A. SCOTT

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NOMENCLATURE AND ABBREVIATIONS

1. Avian erythroid cells

The nomenclature used in this thesis is that of Sadgopal and Kabat (1968).

Cell type	Reference in this thesis	Alternative nomenclature (Lucas and Jamroz, 1961)
Dividing	Erythroblast	Erythroblast
Non-dividing but synthesising RNA and protein	Reticulocyte	Polychromatic erythrocyte
Inactive in macromolecular synthesis	Erythrocyte	Mature erythrocyte

2. Histones

Considerable confusion has arisen from the concurrent use of several systems of histone nomenclature. The system used in this thesis was taken from the CIBA Foundation Symposium on the Structure and Function of Chromatin (Bradbury, 1974). This nomenclature is logical and finding widespread acceptance.

(Histones)

Histone fraction	Ref.a	Ref.b	Ref.c	Ref.d (this thesis)
Lysine-rich	F1	Ia	KAP	1 (H1)
		Ib		
	F2c	V	KAS	5 (H5)
Slightly lysine-rich	F2a2	IIb1	ALK	2A(H2A)
	F2b	IIb2	KSA	2B(H2B)
Arginine-rich	F3	III	ARK	3 (H3)
	F2a1	IV	GRK	4 (H4)

a. Johns (1969, 1971)

b. Fambrough, Fujimura and Bonner (1968)

c. Gordon conference (1972)

d. Bradbury (1974)

3. Abbreviations

mRNA	messenger RNA
rRNA	ribosomal RNA
tRNA	transfer RNA
cDNA	complementary DNA
AMV	avian myeloblastosis virus
OD	optical density (subscript denoting wavelength at which measured)
poly A	polyadenylic acid
BSA	bovine serum albumin
Reverse transcriptase	RNA-dependent DNA-polymerase
Phosphocreatine Kinase	ATP Creatine:phosphotransferase (E.C.2.7.3.2)
Poly A polymerase	ATP Polynucleotidylexotransferase