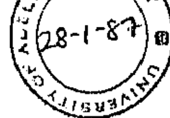
28-1-87

TRANS-STIMULATION OF CHICKEN HISTONE H5 GENE TRANSCRIPTION

A thesis submitted to the University of Adelaide

for the degree of Doctor of Philosophy

by

Peter Lance Wigley B.Sc.(Hons.)

Department of Biochemistry,

University of Adelaide,

Adelaide, South Australia,

Australia

July, 1986

AWARDED 2 OCTOBER, 1986

CONTENTS

CHAPTER 1 : INTRODUCTION — Page

1-1 Control of Gene Expression 1

1-1-1 Levels of Control 1

(1) Transcription 2

(2) RNA processing 2

(3) mRNA stability 2

(4) Translation 3

(5) Post-translational control 3

1-1-2 Transcriptional Control 4

1-2 Active Chromatin 4

1-2-1 DNase I Hypersensitive Sites 5

1-2-2 S1 Hypersensitive Sites and Altered DNA Structures 7

1-2-3 Supercoiling 7

1-2-4 'Open' Nucleosomes 8

1-2-5 Nucleosome Phasing and Nuclear Matrix Association 8

1-2-6 Summary 9

1-3 DNA Sequences Involved in Transcription 10

1-3-1 Prokaryotes 10

1-3-2 Eukaryotes 10

1-3-3 Genes Transcribed by RNA Polymerases I and III 11

(1) RNAP I genes 11

(2) RNAP III genes 12

1-3-4 Genes Transcribed by RNA Polymerase II 12

(1) General initiation elements 12

(2) Common 5' modulator elements 13

(3) Intragenic elements 13

(4) Enhancers 14

(5) Gene-specific elements and Inducible genes 14
(6) Sequences involved in tissue-specific gene expression 15
(7) Negative regulatory regions 17
1-3-5 Summary 18
1-4 Trans-acting Factors 18
1-4-1 Techniques for Identifying Regulatory Factors 19
1-4-2 Prokaryotes 20
1-4-3 Eukaryotic RNAP I and RNAP III Genes 20
(1) RNAP I genes 20
(2) RNAP III genes 21
1-4-4 Eukaryotic RNAP II Genes 22
(1) Yeast 22
(2) Viral transcription factors 23
(3) General transcription factors 24
(4) Enhancer-binding factors 26
(5) Factors for inducible genes 27
(6) Factors involved in tissue-specific gene expression 29
(7) The homeo box 29
1-4-5 Summary 30
1-5 The Xenopus Oocyte System 31
1-5-1 Gene Expression Systems 31
(1) In vitro systems 31
(2) Yeast 32
(3) Cells in culture 32
(4) Transgenic animals 33

1-5-2 Xenopus Oocytes 33

(1) General features 33

(2) Advantages and Limitations 34

(3) Analysis of transcription 35

(4) Co-injection experiments 35

1-6 Histone H5 37

1-6-1 Histone Proteins 37

1-6-2 H5 Protein 39

1-6-3 Histone Genes 41

1-6-4 The Chicken H5 Gene 43

1-7 Aims of this Project 45

CHAPTER 2 : EXPRESSION OF THE CHICKEN H5 GENE IN XENOPUS OOCYTES

2-1 Introduction 46

2-2 Expression of the H5 gene : 5' end mapping of H5 transcripts from chicken erythroid cells and injected oocytes 46

(1) 5' end mapping with RNA from injected oocytes 47

(2) 5' end mapping with RNA from chicken erythroid cells 47

2-3 3' end mapping of H5 transcripts from chicken erythroid cells and injected oocytes 49

(1) 3' end mapping with RNA from chicken erythroid cells 49

(2) 3' end mapping with RNA from injected oocytes 50

2-4 Are chicken H5 transcripts polyadenylated in frog oocytes ? 50

2-5 Cell-type specificity of H5 mRNA in vivo 51

2-6 Discussion 52

CHAPTER 3 : EFFECT OF CHROMATIN SALT-WASH FRACTIONS FROM CHICKEN ERYTHROID CELLS ON H5 TRANSCRIPT LEVELS IN CO-INJECTED OOCYTES

3-1 Introduction 54

3-2 Isolation of chromatin salt-wash fractions 54

3-3 Effect of the CSWFs on H5 transcript levels in co-injected oocytes 56

3-4 Examination of the CSWF effect 58

3-5 Discussion 62

CHAPTER 4 : NATURE OF THE SALT-WASH FRACTION EFFECT: TRANS-STIMULATION OF H5 GENE TRANSCRIPTION

4-1 Introduction 65

4-2 Construction of mH5/H2B 65

4-3 Co-injection of mH5/H2B with CSWFs 67

4-4 Use of the H2B gene in M13 as a control in co-injection experiments 70

4-5 Discussion 71

CHAPTER 5 : REGIONS OF THE H5 GENE INVOLVED IN EFFICIENCY OF TRANSCRIPTION AND TRANS-STIMULATION BY CSWFs

5-1 Introduction 73

5-2 Selection/Construction of 5' deletion mutants of the H5 gene 73

(1) Selection of Bal31 deletion clones 73

(2) Construction of mH5/-395 74

5-3 Effect of the 5' deletions on H5 gene transcription in Xenopus oocytes 75

5-4 Use of 5' deletions of the H5 gene in CSWF co-injection experiments 77

5-5 Construction and functional testing of an H5 gene 3' deletion mutant 77

(1) Construction of the H5 gene 3' deletion clone 78

(2) Use of mH5/-174(delPP) in oocyte experiments 79

5-6 Discussion 80

CHAPTER 6 : INVESTIGATIONS INTO THE NATURE OF THE STIMULATORY FACTOR(S) AFFECTING H5 GENE TRANSCRIPTION

6-1 Introduction 82

6-2 Effect of protein removal on trans-stimulation by the CSWFs 82

6-3 Trans-stimulation with a nucleic acid fraction from AEV cell nuclei 84

(1) Removal of nucleic acids from the CSWFs 85

(2) Testing of a nucleic acid fraction from AEV cell nuclei 86

6-4 Investigation of the cell-type specificity of the stimulatory factor(s) 88

6-5 Discussion 89

CHAPTER 7 : EFFECT OF H5 PROTEIN ON TRANSCRIPTION IN CO-INJECTED OOCYTES

7-1 Introduction 92

7-2 Effect of H5 protein on transcription from co-injected DNA 93

7-3 Characterisation of the UH2B band 95

7-4 Examination of the H5 protein effect 97

7-5 Discussion 99

CHAPTER 8 : FINAL DISCUSSION

8-1 Conclusions and Discussion 102

8-2 Future work 106

CHAPTER 9 : MATERIALS AND METHODS

9-1 Abbreviations 108

9-2 Materials 108

9-2-1 Chemicals and Reagents 108

9-2-2 Enzymes 109

9-2-3 Bacterial media 109

9-2-4 Bacterial strains and cloned DNA sequences 110

9-3 Methods 110

9-3-1 Isolation of plasmid DNA 110

(i) Growth and amplification of plasmid DNA 110

(ii) Large scale isolation 111

(iii) Miniscreen procedure 112

9-3-2 Isolation of M13 replicative form 112

9-3-3 Restriction endonuclease digestions 112

9-3-4 Routine gel electrophoresis 113

(i) Polyacrylamide gel electrophoresis 113

(ii) Agarose gel electrophoresis 114

(iii) Low melting point agarose gel electrophoresis 114

9-3-5 Autoradiography 115

9-3-6 Elution of DNA from polyacrylamide gels 115

9-3-7 Isolation of DNA from low melting point agarose 116

9-3-8 Subcloning of DNA fragments into plasmid and M13 vectors 116

(i) End-filling of DNA fragments and blunt-end ligations 116

(ii) Sticky-end ligations 117

9-3-9 Transformation procedure for plasmid recombinants 117

9-3-10 Transformation procedure for M13 recombinants 117

9-3-11 M13 chain terminator sequencing of DNA 118

(i) Preparation of template 118

(ii) Sequencing reactions 118

(a) Hybridisation 119

(b) Polymerisation 119

(iii) Sequencing gels 120

9-3-12 'Kinasing' of synthetic oligonucleotides 120

9-3-13 Nick translation of DNA 121

9-3-14 Injection of Xenopus oocytes 122

(i) Animals 122

(ii) Frog dissection 122

(iii) Oocytes 122

(iv) Components of the injection system 123

(v) Micro-injection technique 123

(a) Filling of needle 123

(b) Preparation of DNA for injection 123

(c) Nuclear injection 124

(d) Co-injection 124

(vi) Modified Barths' saline 125

9-3-15 Cell culture 125

9-3-16 Isolation of chromatin salt-wash fractions 126

(i) Nuclei isolation 126

(ii) Chromatin isolation 126

(iii) Isolation of salt-wash fractions 126

9-3-17 Isolation of Xenopus oocyte and cultured cell RNA 127

(i) Xenopus oocyte RNA 127

(ii) Cultured cell RNA 127

9-3-18 Separation of poly A^+ and poly A^- RNA on poly U-sepharose 128

9-3-19 Northern hybridisation analysis of RNA 128

9-3-20 S1 nuclease analysis of RNA 129

9-3-21 Primer extension analysis of RNA 131

9-3-22 Containment facilities 133

BIBLIOGRAPHY 133

THESIS SUMMARY

Expression analysis was carried out with the gene for the erythroid-specific chicken histone H5. The primary aim of this work was to identify trans-acting factors involved in the transcription of this gene. The Xenopus oocyte was chosen as the assay system for this study, since cell extracts can be co-injected, with DNA, into oocytes, and since it had previously been shown to be useful in the identification of regulatory factors. The results obtained in this study are outlined below.

(1) Transcription of the H5 gene was shown to be accurately initiated in frog oocytes, and parameters of H5 gene expression were defined.

(2) 5' deletions of the H5 gene were used to identify upstream regions involved in determining transcriptional efficiency in injected oocytes. Two such regions were identified - one which inhibited transcription and one which was involved in stimulation of transcription.

(3) In order to identify H5 gene transcription factors, chromatin salt-wash fractions (CSWFs), made from chicken erythroid cells (a transformed cell line, grown in culture) were co-injected, with the H5 gene, into frog oocytes. Other chicken histone genes (H1, H2A and H2B) were also injected in these experiments, to act as internal controls. Primer extension analysis on RNA from injected oocytes indicated that the CSWFs produce an increase in the level of H5 gene transcripts, relative to the transcripts from the control genes.

(4) The H2B gene was cloned next to the H5 gene, in M13. Co-injection of this clone with the CSWFs (and control genes) resulted in an increase in the level of both the H5 and the H2B transcripts. This, together with subsequent experiments, suggested that the CSWFs stimulate H5 gene transcription, and that this effect involves an enhancer-like activity. Furthermore, this effect appears to be mediated by H5 gene sequences.

(5) As an initial investigation into the regions of the H5 gene involved in the trans-stimulation effect, 5' and 3' deletions of the H5 gene were used in CSWF co-injection experiments. The results suggest that a region of the H5 gene between -85 and +313 (relative to the cap site at +1) is sufficient to generate trans-stimulation of H5 gene transcription.

(6) A preliminary study was undertaken into the nature of the stimulatory factor(s) present in the CSWFs. Firstly, treatment of the CSWFs with proteinase K and phenol/chloroform extraction did not reduce the ability of the CSWFs to stimulate H5 gene transcription. Secondly, a nucleic acid fraction from chicken erythroid cell nuclei was also shown to stimulate H5 gene transcription in co-injected oocytes. Finally, treatment of the nucleic acid fraction with RNase reduced the capacity of this fraction to stimulate H5 transcription. This suggests that the stimulatory factor identified in these experiments may be an RNA molecule, or an RNA-protein complex. [This RNA cannot be the H5 mRNA from the chicken erythroid cells since this has an extra 9 bases in the 5' untranslated region, compared with the transcript produced in injected oocytes, due to a polymorphic insertion/deletion.]

(7) A nucleic acid fraction from chicken T cell nuclei was also found to stimulate H5 transcription in co-injected oocytes, suggesting that the stimulatory factor may not be erythroid cell-specific.

Finally, in addition to the work described above, a study was also undertaken to examine some of the functional properties of H5 protein. Co-injection of H5 protein into oocytes, with plasmids containing three chicken histone genes, resulted in the inhibition of transcription from a cryptic RNA polymerase II promoter located within the histone gene cluster.