



<http://researchspace.auckland.ac.nz>

### *ResearchSpace@Auckland*

#### **Copyright Statement**

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

To request permissions please use the Feedback form on our webpage.

<http://researchspace.auckland.ac.nz/feedback>

#### **General copyright and disclaimer**

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form.

Regulation of the beta7  
integrin gene in T cells-  
Role of the MAPK  
signalling pathways

**Farhad Shafiei**

Department of Molecular Medicine & Pathology

A Thesis submitted for the degree of Doctor of  
Philosophy at The University of Auckland,  
Auckland, New Zealand

October 2004

*To my dear parents, for without their love and support, this would not  
have been possible.*

Thesis  
W4  
S525  
2004  
c.2

# Abstract

Members of the integrin superfamily of adhesion molecules are involved in cell to cell, cell to ECM, and cell to pathogen interactions, and are of fundamental importance in many biological processes. The  $\beta 7$  integrins  $\alpha 4\beta 7$  and  $\alpha E\beta 7$  have evolved to play specialized roles in gut mucosal immunity.  $\alpha 4\beta 7$  mediates the homing of lymphocytes to intestinal Peyer's patches, mesenteric lymph nodes, and the lamina propria by binding to the vascular addressin MAdCAM-1 (Fong *et al.*, 1997).  $\alpha E\beta 7$  binds epithelial E-cadherin retaining lymphocytes at the intraepithelial compartment of the mucosa. The expression of  $\alpha E\beta 7$  is induced on migratory lymphocytes by TGF- $\beta$  secreted by gut enterocytes. The signalling mechanisms responsible for basal and TGF- $\beta$ -induced expression of  $\beta 7$  integrins are not well understood. Previous studies identified two TGF- $\beta 1$  response regions in the  $\beta 7$  gene promoter termed TGFBR-1 and TGFBR-2 encompassing nucleotides -509 to -398 and -121 to -34. Here, TGF- $\beta 1$  activation of the  $\beta 7$  gene proximal promoter is shown to be mediated by MAPK family members. TGF- $\beta 1$  stimulation of TK-1 T cells increased the steady-state mRNA levels of the  $\beta 7$  gene relative to  $\alpha 4$  transcripts, and led to enhanced phosphorylation of c-Jun. TGF- $\beta 1$  stimulation induced rapid increases in the binding of nuclear protein complexes to TGFBR-1 and -2. Sp1 and Sp3 which mediate TGF- $\beta 1$  signalling were shown to bind to an Sp1 cis-element encompassing nucleotide positions -67 to -60 within TGFBR-2. The interaction of Sp1 with its cognate binding site was c-Jun dependent. In contrast, there was no evidence for involvement of the Smad proteins. ATF-2 was identified to bind to a region encompassing nucleotide positions -699 to -689 just upstream of TGFBR-1. Sp1 and ATF-2 expression vectors co-transfected into Sp1-deficient SL-2 cells synergized to drive the activity of a  $\beta 7$  gene luciferase reporter. Mutation of the ATF-2-binding site modestly reduced  $\beta 7$  gene reporter activity.

The involvement of c-Jun in TGF- $\beta$  signalling and interaction of Sp1 with the  $\beta$ 7 gene promoter suggested that MAP kinase pathways might mediate  $\beta$ 7 gene transcription. Specific chemical inhibitors were used to ascertain which of the three MAPK pathways namely p38, JNK, and ERK were involved. Results obtained by nuclear run-on transcription analysis which measures nascent RNA synthesis showed that both the p38 and JNK pathways regulate  $\beta$ 7 gene expression in TK-1 cells, whereas only the p38 pathway regulates  $\alpha$ 4 gene expression. Thus, treatment of TK-1 cells with the p38 inhibitor SB203580 and the JNK inhibitor SP600125 inhibited the synthesis of  $\beta$ 7 transcripts, whereas only SB203580 inhibited the synthesis of  $\alpha$ 4 transcripts. Conversely, sodium arsenite which induces JNK and p38 upregulated nascent synthesis of  $\alpha$ 4 and  $\beta$ 7 RNA transcripts. SB203580 blocked the binding of nuclear factors to TGFBR-1, and ATF-2 binding to nucleotide position -699 to -689. Similarly, SP600125 blocked the binding of Sp1 and Sp3 to TGFBR-2, whereas unexpectedly SB203580 enhanced their binding. Furthermore, both SB203580 and SP600125 decreased cell-surface expression of the  $\beta$ 7 subunit and SB203580 inhibited TK-1 cell adhesion to MAdCAM-1. In contrast, the MEK inhibitor PD98059 had no effect on the expression of nascent  $\beta$ 7 RNA transcripts and cell-surface expression of the  $\beta$ 7 subunit, suggesting that the ERK pathway is not involved in regulation of  $\beta$ 7 gene expression in TK-1 cells. In contrast to the results obtained with TK-1 cells, SB203580, SP600125, and PD98059 each inhibited the nascent synthesis of  $\alpha$ 4,  $\beta$ 7, and  $\alpha$ E transcripts in peripheral blood lymphocytes.

In conclusion, this study has revealed for the first time that both the p38 and JNK pathways mediate TGF- $\beta$ 1-induced expression of the integrin  $\beta$ 7 gene. Expression of the  $\beta$ 7 gene is Sp1-dependent, and involves the synergistic cooperation of c-Jun and ATF-2. It is proposed that the p38 and JNK pathways play a role by triggering the activation and translocation of c-Jun and ATF-2 to the nucleus.

# Acknowledgements

First and foremost, I would like to express my gratitude to my supervisor Associate Professor Geoffrey Krissansen for all his guidance, advice, and support throughout the course of this study. He has been a great mentor, and his patience, positive attitude, and work ethic have taught me a great deal at a personal level. I would also like to thank my advisor Dr Euphemia Leung who has a wealth of technical knowledge in the field and my colleague Mr Sushil Pandey for many helpful discussions and the opportunity to present two relevant results from his work namely Figures 3.6 and 3.8 in this thesis. I would like to thank Ms Yi Yang for preparation of recombinant cell adhesion molecules and help with the FACS analysis. My gratitude also extends to other laboratory colleagues including Dr Lidija Petreska, Dr Klaus Lehnert, Dr Jagat Kanwar, Dr Rupinder Kanwar, Dr Ji Zhong Bai, Mr Yih Chih Chan, and Mrs Rita Gupta for many helpful discussions and suggestions.

This work is dedicated to my parents Mr Karim Shafiei and Mrs Bitra Shafiei for many reasons. I am grateful to them for all their love, support, and encouragement throughout my studies. They are and always have been my role models in life. I would like to thank my brother Mr Farzad Shafiei for his unconditional support and for the knowledge and expertise provided in the preparation of this thesis. I am indebted to the Marsden Fund for providing the necessary funding for the project, and the Maurice and Phyllis Paykel Trust for the opportunity to present my findings at two international conferences.

# Table of Contents

<b>Abstract</b> .....	<b>I</b>
<b>Acknowledgements</b> .....	<b>III</b>
<b>Table of Contents</b> .....	<b>IV</b>
<b>List of Figures</b> .....	<b>IX</b>
<b>List of Tables</b> .....	<b>XI</b>
<b>Abbreviations</b> .....	<b>XII</b>
<b>Chapter 1: Introduction</b> .....	<b>1</b>
<b>1.1 Integrins</b> .....	<b>1</b>
<b>1.2 Structural features of integrins</b> .....	<b>2</b>
1.2.1 The $\alpha$ - subunit .....	4
1.2.2 The $\beta$ -subunit.....	6
<b>1.3 Integrins and their ligands</b> .....	<b>7</b>
<b>1.4 Integrins are signalling molecules</b> .....	<b>9</b>
1.4.1 "Inside-out" signalling.....	9
1.4.2 Small GTP-binding proteins .....	10
1.4.2.1 Ras GTPases .....	10
1.4.2.2 RhoA and Rac1 .....	11
1.4.3 "Outside-in" signalling .....	12
1.4.3.1 Rho family .....	12
1.4.3.2 Protein tyrosine kinases .....	12
1.4.3.3 Fyn and Shc.....	13
<b>1.5 The <math>\beta 7</math> integrins</b> .....	<b>14</b>
1.5.1 Regulation and functions of $\beta 7$ integrins.....	14
1.5.1.1 $\alpha 4\beta 7$ .....	14
1.5.1.2 $\alpha E\beta 7$ .....	15
1.5.1.3 The role of $\beta 7$ integrin subunits in ligand recognition.....	15
<b>1.6 Integrins and disease</b> .....	<b>16</b>
1.6.1 Hereditary diseases .....	16
1.6.2 Integrins are receptors for microbes and viruses.....	16
1.6.3 Integrins and inflammatory diseases.....	17
1.6.3.1 Integrin $\alpha 4$ antagonists.....	18
1.6.4 The role of $\beta 7$ integrins in disease .....	19
1.6.5 Integrins and cancer .....	20
<b>1.7 Mechanisms of transcription</b> .....	<b>21</b>
1.7.1 The general transcription factors .....	21
1.7.2 The specific transcription factors.....	23
1.7.3 Sp1 is a key activator of TATA-less promoters.....	25
1.7.4 The $\beta 7$ integrin gene promoter .....	26
<b>1.8 The transforming growth factor-<math>\beta</math> family</b> .....	<b>28</b>
1.8.1 TGF- $\beta$ receptors.....	30
1.8.2 Smads: Mediators of TGF- $\beta$ signal transduction.....	30
1.8.3 Other mediators of TGF- $\beta$ signalling.....	31
<b>1.9 Mitogen activated protein kinases (MAPKs)</b> .....	<b>32</b>

1.9.1 Cross-talk between Smads and MAPKs .....	36
1.9.2 The ERK pathway.....	37
1.9.2.1 The MEK inhibitors, PD98059 and UO126.....	37
1.9.3 The JNK pathway .....	38
1.9.3.1 The JNK inhibitor, SP600125 .....	39
1.9.4 The p38 pathway.....	39
1.9.4.1 The p38 inhibitor, SB203580.....	40
<b>1.10 TGF-<math>\beta</math> signalling that is independent of Smads .....</b>	<b>40</b>
<b>1.11 TGF-<math>\beta</math>1 induces gene expression in an Sp1-dependent fashion .....</b>	<b>42</b>
<b>1.12 The project.....</b>	<b>43</b>
<b>Chapter 2: Materials &amp; Methods .....</b>	<b>44</b>
<b>2.1 Common buffers.....</b>	<b>44</b>
<b>2.2 Common reagents.....</b>	<b>46</b>
2.2.1 Chemicals and solvents.....	46
2.2.2 Polymerase chain reaction (PCR) .....	46
2.2.3 Oligonucleotides .....	46
2.2.4 Enzymes.....	48
2.2.5 Antibodies.....	48
2.2.6 Liquid media.....	48
2.2.7 Agarose.....	49
2.2.8 DNA and protein markers.....	49
2.2.9 Ethidium bromide (EtBr).....	49
<b>2.3 Commercial kits.....</b>	<b>49</b>
<b>2.4 Cloning vectors .....</b>	<b>49</b>
<b>2.5 DNA sequencing .....</b>	<b>50</b>
<b>2.6 Sequence analysis .....</b>	<b>50</b>
<b>2.7 Radioactive isotopes .....</b>	<b>50</b>
<b>2.8 Recombinant proteins.....</b>	<b>51</b>
<b>2.9 Inhibitors.....</b>	<b>51</b>
<b>2.10 Slides.....</b>	<b>52</b>
<b>2.11 IMAGE Clones .....</b>	<b>52</b>
<b>2.12 Cell cultures .....</b>	<b>52</b>
2.12.1 Culture of mouse TK-1 cells.....	52
2.12.2 Culture of <i>Drosophila</i> Schneider SL-2 cells.....	52
<b>2.13 Isolation of human peripheral blood lymphocytes (PBLs).....</b>	<b>53</b>
<b>2.14 Polymerase chain reaction (PCR).....</b>	<b>54</b>
2.14.1 Purification of PCR products.....	54
<b>2.15 DNA cloning.....</b>	<b>55</b>
2.15.1 Digestion of DNA with restriction enzymes.....	55
2.15.2 Ligation of DNA inserts into plasmid vectors .....	55
2.15.2.1 Preparation of vector and insert .....	55
2.15.2.2 Ligation.....	56
2.15.3 Ligation of PCR products into pGEM®-T.....	56
2.15.4 Ligation of PCR products into pMT/V5-His-TOPO.....	57
2.15.5 Preparation of competent <i>E.coli</i> cells using rubidium chloride (RbCl) .....	57
2.15.6 Transformation of competent DH5 $\alpha$ bacteria.....	58
<b>2.16 DNA extractions .....</b>	<b>58</b>



2.16.1 Small scale extraction of plasmid DNA.....	58
2.16.2 Large scale extraction of plasmid DNA using cesium chloride (CsCl) .....	59
2.16.3 Extraction of DNA from agarose gels.....	60
<b>2.17 Calcium phosphate-mediated transfection of SL-2 cells.....</b>	<b>60</b>
2.17.1 $\beta$ -galactosidase assays .....	61
2.17.2 Luciferase gene reporter assays .....	61
<b>2.18 Electromobility shift assay (EMSA) .....</b>	<b>62</b>
2.18.1 Preparation of nuclear extracts.....	62
2.18.2 Preparation of EMSA probes.....	63
2.18.3 Purification of labelled probes by polyacrylamide gel electrophoresis (PAGE).....	64
2.18.4 Preparation of competitor probes.....	64
2.18.5 Electromobility shift assay (EMSA).....	65
<b>2.19 RNA extractions .....</b>	<b>65</b>
<b>2.20 Nuclear run-on transcription assay .....</b>	<b>66</b>
2.20.1 Preparation of membrane blots .....	66
2.20.2 Isolation and labelling of nascent RNA transcripts.....	66
2.20.2.1 Isolation of nuclei.....	66
2.20.2.2 Labelling of newly transcribed RNA .....	67
2.20.3 Hybridisation and development of blots .....	67
<b>2.21 Northern blot analysis.....</b>	<b>68</b>
2.21.1 Transfer of RNA from gel to membrane.....	68
2.21.2 Preparation of cDNA probes.....	68
2.21.3 Hybridisation of blots .....	69
<b>2.22 Flow cytometry analysis.....</b>	<b>69</b>
<b>2.23 Cell adhesion assays .....</b>	<b>70</b>
<b>2.24 In-vitro kinase assay.....</b>	<b>70</b>
<b>Chapter 3: Results .....</b>	<b>72</b>
3.1 TGF- $\beta$ 1 induces transcription of the $\beta$ 7 gene .....	72
3.2 TGF- $\beta$ 1 induces the expression of nascent $\beta$ 7 RNA transcripts .....	75
3.3 TGF- $\beta$ 1 has no effect on the overall levels of cell-surface $\beta$ 7 integrins, but combines with PMA to induce expression of $\alpha$ E $\beta$ 7 .....	77
3.4 The integrin $\beta$ 7 gene promoter is responsive to TGF- $\beta$ 1 .....	79
3.5 Identification of potential cis-elements in the integrin $\beta$ 7 gene promoter .....	81
3.6 Sp1 and Sp3 transcription factors bind to TGFBR-2 .....	82
3.7 Sp1 binding to an Sp1 cis-element within TGFBR-2 is enhanced by TGF- $\beta$ 1....	83
3.8 Binding of Sp1 to its cognate binding site within TGFBR-2 is dependent on c- Jun .....	84
3.9 TGF- $\beta$ 1 stimulation of cells leads to the phosphorylation of c-Jun .....	85
3.10 Four potential Smad-binding sites are responsive to TGF- $\beta$ 1 .....	86
3.10.1 Smad binding is not observed at sites -719 and -623 .....	87
3.10.2 Smad binding is not observed at sites -354 and -216 whereas Sp1 binds to site -354....	89
3.11 ATF-2 binds to a putative Sp1-binding site within the integrin $\beta$ 7 gene promoter .....	90
3.11.1 ATF-2 binding is inhibited by a consensus ATF-2 cis-element competitor .....	92
3.12 Defining the MAPK pathways that control $\beta$ 7 gene transcription .....	94
3.12.1 The p38 inhibitor SB203580 blocks the binding of nuclear factors to TGFBR-1 .....	94

3.12.2 The p38 inhibitor SB203580 abolishes the binding of ATF-2 to its binding site within the integrin $\beta 7$ promoter .....	96
3.12.3 Opposing effects of SB203580 and SP600125 on the binding of Sp1 and Sp3 transcription factors to TGFBR-2 .....	98
<b>3.13 The MAPK family of protein kinases mediates TGF-<math>\beta 1</math> upregulation of integrin <math>\alpha 4</math> and <math>\beta 7</math> gene expression .....</b>	<b>100</b>
3.13.1 The p38 inhibitor SB203580 downregulates integrin $\alpha 4$ and $\beta 7$ gene transcription .....	100
3.13.2 The JNK inhibitor SP600125 specifically downregulates $\beta 7$ gene transcription.....	101
3.13.3 The MEK inhibitor PD98059 has no effect on integrin $\alpha 4$ and $\beta 7$ gene transcription ....	102
<b>3.14 Sodium arsenite upregulates <math>\alpha 4</math> and <math>\beta 7</math> transcription.....</b>	<b>104</b>
<b>3.15 Modulation of integrin <math>\beta 7</math> subunit expression at the cell-surface by MAPK inhibitors .....</b>	<b>105</b>
3.15.1 Blockade of the p38 and JNK pathway significantly reduces cell-surface basal expression of the integrin $\beta 7$ subunit.....	105
<b>3.16 The effect of MAPK inhibitors on <math>\alpha 4\beta 7</math>-mediated cell adhesion to MAdCAM-1 .....</b>	<b>107</b>
3.16.1 Prolonged blockade of the p38 pathway inhibits cell adhesion to MAdCAM-1 .....	107
3.16.2 Short-term blockade of the p38 pathways does not effect cell binding to MAdCAM-1..	108
<b>3.17 The effect of the MAPK inhibitors on <math>\alpha 4</math>, <math>\beta 7</math>, and <math>\alpha E</math> gene expression in human peripheral blood lymphocytes .....</b>	<b>109</b>
3.17.1 Blockade of the p38 pathway inhibits $\beta 7$ gene transcription .....	110
3.17.2 Blockade of the JNK pathway inhibits $\beta 7$ gene transcription.....	110
3.17.3 Blockade of the ERK pathway inhibits $\beta 7$ gene transcription .....	110
<b>3.18 Sp1 is a key regulator of integrin <math>\beta 7</math> gene expression .....</b>	<b>112</b>
3.18.1 Transfection of SL-2 cells with the $\beta$ -galactosidase gene.....	114
3.18.2 Sp1 drives the activity of the integrin $\beta 7$ gene promoter.....	115
<b>3.19 ATF-2 synergizes with Sp1 to drive <math>\beta 7</math> gene expression .....</b>	<b>117</b>
3.19.1 Engineering ATF-2-deficient SL-2 cells to express exogenous ATF-2.....	117
3.19.2 ATF-2 by itself only weakly drives the expression of the $\beta 7$ gene reporter .....	119
<b>3.20 ATF-2 combines with Sp1 to enhance the expression of the <math>\beta 7</math> gene reporter ..</b>	<b>121</b>
<b>3.21 Mutation of the ATF-2 binding site modestly reduces <math>\beta 7</math> gene reporter activity .....</b>	<b>123</b>
<b>Chapter 4: Discussion.....</b>	<b>125</b>
<b>4.1 The <math>\beta 7</math> gene promoter resembles the <math>\beta 2</math> and CD11b integrin gene promoters ...</b>	<b>125</b>
<b>4.2 Transcription factors that bind the <math>\beta 7</math> gene promoter .....</b>	<b>126</b>
4.2.1 Sp1 binds to multiple sites in the $\beta 7$ gene promoter .....	127
4.2.2 Sp3 binds to five sites in the $\beta 7$ gene promoter .....	128
4.2.3 Sp1 binding is facilitated by c-Jun.....	128
4.2.4 ATF-2 binds to a site upstream of TGFBR-1 .....	129
<b>4.3 Sp1 regulates integrin <math>\beta 7</math> gene expression .....</b>	<b>130</b>
4.3.1 ATF-2 and c-Jun each combine with Sp1 to enhance integrin $\beta 7$ gene promoter activity	130
4.3.2 Sp3 suppresses Sp1-mediated $\beta 7$ gene expression .....	131
<b>4.4 TGF-<math>\beta 1</math> induces <math>\beta 7</math> gene transcription .....</b>	<b>132</b>
4.4.1 The role of TGF- $\beta 1$ in $\alpha 4$ gene transcription remains undefined .....	132
<b>4.5 The role of TGF-<math>\beta 1</math> in the expression of integrins <math>\alpha 4\beta 7</math> and <math>\alpha E\beta 7</math> .....</b>	<b>133</b>
4.5.1 $\alpha E$ subunit expression on the surface of TK-1 cells .....	133
4.5.2 Cell-surface levels of the $\beta 7$ subunit are unaffected by TGF- $\beta 1$ stimulation .....	134

<b>4.6 Lineage-restricted distributions of <math>\alpha 4\beta 7</math> and <math>\alpha E\beta 7</math> .....</b>	<b>135</b>
<b>4.7 Signalling mechanisms mediating TGF-<math>\beta</math>1-induced <math>\beta 7</math> gene expression .....</b>	<b>136</b>
4.7.1 The p38 pathway mediates TGF- $\beta$ 1-induced $\beta 7$ gene expression in TK-1 cells .....	136
4.7.2 JNK contributes to TGF- $\beta$ 1-induced $\beta 7$ gene expression in TK-1 cells.....	137
4.7.3 The ERK pathway may contribute TGF- $\beta$ 1-induced $\beta 7$ gene expression in normal T-cells .....	138
<b>4.8 The specificity of the MAPK inhibitors.....</b>	<b>139</b>
<b>4.9 TGF-<math>\beta</math>1-mediated upregulation of <math>\beta 7</math> gene expression appears to be independent of Smad signalling .....</b>	<b>140</b>
<b>4.10 Other modulators of <math>\beta 7</math> expression.....</b>	<b>142</b>
4.10.1 MAPKs play an important role in TNF-mediated signalling.....	142
<b>4.11 Blockade of the p38 pathway inhibits <math>\beta 7</math> integrin-mediated T cell adhesion to MAdCAM-1 .....</b>	<b>143</b>
<b>4.12 A signalling model for TGF-<math>\beta</math>1-induced <math>\beta 7</math> gene regulation .....</b>	<b>144</b>
<b>4.14 Future directions .....</b>	<b>147</b>
<b>References.....</b>	<b>149</b>

# List of Figures

Figure 1.1: Integrin subunits and pairings. ....	2
Figure 1.2: Integrin architecture. ....	4
Figure 1.3: Schematic illustrations of the structures of $\alpha V\beta 3$ . ....	10
Figure 1.4: Models of the (A) FAK, and (B) Shc pathways. ....	13
Figure 1.5: The formation of a pre-initiation complex in mammalian cells. ....	22
Figure 1.6: A model of mediator function. ....	24
Figure 1.7: DNA sequence of the promoter of the mouse $\beta 7$ subunit gene. ....	27
Figure 1.8: Parallel MAPK cascades involve specific MAPK enzyme modules. ....	33
Figure 3.1: TGF- $\beta 1$ increases the steady-state levels of integrin $\beta 7$ mRNA transcripts. ....	74
Figure 3.2: TGF- $\beta 1$ induces the expression of nascent $\beta 7$ RNA transcripts relative to $\alpha 4$ transcripts. ....	76
Figure 3.3: TGF- $\beta 1$ has no effect on the level of cell-surface integrin $\beta 7$ expression but combines with PMA to induce $\alpha E$ expression. ....	78
Figure 3.4: Enhanced binding of nuclear proteins to TGFBR-1 and TGFBR-2 upon stimulation with TGF- $\beta 1$ . ....	80
Figure 3.5: Multiple potential transcription factor binding sites within the integrin $\beta 7$ gene proximal promoter. ....	81
Figure 3.6: Sp1 and Sp3 bind to TGFBR-2. ....	82
Figure 3.7: Enhanced binding of Sp1 to the Sp1 cis-element starting at nucleotide position -67 within TGFBR-2. ....	83
Figure 3.8: The interaction of Sp1 with its cognate binding site is c-Jun-dependent. ....	84
Figure 3.9: TGF- $\beta 1$ causes rapid and transient increases in the level of phosphorylation of c-Jun. ....	85
Figure 3.10: Enhanced binding of nuclear proteins to four potential Smad binding sites in response to TGF- $\beta 1$ . ....	87
Figure 3.11: Supershift assay of potential Smad binding sites starting at nt positions -719 & -623 fails to identify Smads as components of the DNA-protein complexes. ....	88
Figure 3.12: Supershift assay of potential Smad binding sites starting at nucleotides -354 & -216 reveals Sp1 binds to the site at -354. ....	90
Figure 3.13: A putative Sp1-binding site starting at nucleotide position -699 within the proximal $\beta 7$ gene promoter binds ATF-2. ....	91
Figure 3.14: Binding of ATF-2 to the putative Sp1 binding site starting at nucleotide position -699 is inhibited by an ATF-2 oligonucleotide. ....	93
Figure 3.15: Binding of nuclear proteins to TGFBR-1 is inhibited by blockade of the p38 pathway. ....	95
Figure 3.16: SB203580 and an anti-ATF-2 antibody inhibit the binding of ATF-2 to its binding site starting at nucleotide position -699. ....	97
Figure 3.17: Binding of Sp1 and Sp3 to TGFBR-2 is inhibited by SP600125 but enhanced by SB203580. ....	99
Figure 3.18: SB203580 downregulates $\alpha 4$ and $\beta 7$ gene transcription in TK-1 cells. ....	101
Figure 3.19: SP600125 specifically blocks $\beta 7$ gene transcription in TK-1 cells. ....	102
Figure 3.20: PD98059 has no effect on $\alpha 4$ and $\beta 7$ gene transcription in TK-1 cells. ....	103
Figure 3.21: Arsenite upregulates $\alpha 4$ and $\beta 7$ gene transcription. ....	104

Figure 3.22: SB203580 and SP600125 decrease the basal cell-surface expression of the integrin $\beta 7$ subunit on TK-1 cells. ....	106
Figure 3.23: Prolonged blockade of the p38 pathway inhibits $\alpha 4\beta 7$ -mediated adhesion of TK-1 cells to MAdCAM-1. ....	108
Figure 3.24: Short-term blockade of the p38 and MEK pathways has no apparent effect on the adhesion of TK-1 cells to MAdCAM-1. ....	109
Figure 3.25: MAPK inhibitors decrease $\alpha 4$ , $\beta 7$ , and $\alpha E$ gene transcription in peripheral blood lymphocytes. ....	111
Figure 3.26: Engineering Sp1-deficient SL-2 cells to express exogenous Sp1. ....	113
Figure 3.27: Sp1 by itself can drive the expression of a $\beta 7$ gene reporter. ....	116
Figure 3.28: Engineering SL-2 cells to overexpress ATF-2. ....	118
Figure 3.29: ATF-2 alone only weakly stimulates the activity of the integrin $\beta 7$ gene promoter. ....	120
Figure 3.30: ATF-2 and Sp1 synergize to drive expression of the $\beta 7$ gene reporter. ....	122
Figure 3.31: Mutation of the ATF-2-binding site at nt positions -699 to -689 modestly reduces the activity of the $\beta 7$ gene reporter in response to Sp1 and ATF-2. ....	124
Figure 4.1: A proposed signal transduction model for TGF- $\beta 1$ -induced expression of the $\beta 7$ gene in TK-1 cells. ....	146

# List of Tables

Table 1.1: The integrin superfamily: Distribution and ligands. ....	8
Table 1.2: Microbial ligands for integrins. ....	17
Table 1.3: The TGF- $\beta$ superfamily and their representative functions. ....	29
Table 1.4: Enzymes involved in the MAP Kinase signalling pathways.....	35
Table 2.1: Sequence of oligonucleotide primers used to amplify the full-length $\beta$ 7, GAPDH, Smad4, and ATF-2 cDNAs.....	46
Table 2.2: Sequence of oligonucleotides representing different regions within the proximal $\beta$ 7 gene promoter. ....	47
Table 3.1: Determination of the amount of pSV- $\beta$ -galactosidase control vector required to obtain optimal transfection of SL-2 cells. ....	114
Table 3.2: The wild-type and mutated ATF-2 sequences at nt positions -699 to -689. .....	123

# Abbreviations

A	absorbance
Act D	actinomycin D
ATP	adenosine triphosphate
AMP <sup>r</sup>	ampicillin resistance
ATCC	American Type Culture Collection
bp	base pair
cDNA	complementary DNA
Ci	curies
©	copyright
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
dNTP	deoxynucleoside triphosphate
dTTP	deoxythymidine triphosphate
DEPC	diethylpyrocarbonate
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DTT	dithiothreitol
ECM	extracellular matrix
EDTA	ethylenediaminetetra-acetic acid
ERK	extracellular signal-regulated protein kinase
EtBr	ethidium bromide
FACS	fluorescence-activated cell sorter
FMLP	f-methionine-leucine-phenylalanine
FN	fibronectin
fpu	foot print unit
G3	glyceraldehyde-3-phosphate dehydrogenase
h	hour
HBS	hepes-buffered saline
HEV	high endothelial venules
HBSS	Hanks balanced salt solution
ICAM-1	intercellular adhesion molecule-1
IFN- $\gamma$	interferon- $\gamma$
IEL	intraepithelial lymphocytes
IL	interleukin
IMAGE	integrated molecular analysis of genomes and their expression
JNK	c-Jun N-terminal kinase
kb	kilobase
kDa	kilodalton

L	litre
LB	luria-bertani
LCPS	luminescent counts per second
LPAM-1	lymphocyte Peyer's patch adhesion molecule-1
MAdCAM-1	mucosal addressin cell adhesion molecule-1
μ	micro
m	milli
M	molar
MAPK	mitogen activated protein kinase
MOPS	morpholinol propane sulfonic acid
ml	millilitre(s)
μl	microlitre(s)
mM	millimolar
mAb	monoclonal antibody
mRNA	messenger RNA
min	minute(s)
NAD	nicotinamide adenine dinucleotide
NK	natural killer
ng	nanogram(s)
nm	nanometre(s)
nt	nucleotide
nM	nanomole(s)
pM	picomole(s)
P	p-value
PBS	phosphate-buffered saline
PKC	protein kinase C
PMA	phorbol 12-myristate 13-acetate
PMSF	phenylmethyl sulphonyl fluoride
RGD	arginine-glycine-asparagine
rh	recombinant human
RT	room temperature
RNA	ribonucleic acid
rRNA	ribosomal RNA
Rpm	revolutions per minute
®	registered
S	second
SDS	sodium dodecyl sulphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SL-2	<i>Drosophila</i> Schneider cell line
TAE	tris-acetate-EDTA
TAB1	TAK1-binding protein 1
TAB2	TAK1-binding protein 2



TAK1	TGF- $\beta$ activated kinase 1
TBE	tris-borate-EDTA
TEMED	tetramethylethylenediamine
TfbI	transformation buffer I
TfbII	transformation buffer II
TGF- $\beta$	transforming growth factor- $\beta$
TNF- $\alpha$	tumour necrosis factor- $\alpha$
U	unit(s)
UTP	uridine triphosphate
UV	ultraviolet
V	volt(s)
v	volume
VCAM-1	vascular cell adhesion molecule-1
w	weight
WWW	world wide web