MOLECULAR INTERACTIONS OF LATENT TRANSFORMING GROWTH FACTOR–β BINDING PROTEIN–2 (LTBP–2) WITH FIBRILLINS AND OTHER EXTRACELLULAR MATRIX MACROMOLECULES: LTBP–2 COMPETES WITH LTBP–1 FOR BINDING TO FIBRILLIN–1 SUGGESTING THAT LTBP–2 MAY MODULATE LATENT TGF–β STORAGE

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Submitted for the degree of Doctor of Philosophy (PhD) in August, 2006 with permission from the Faculty of Health Sciences, the University of Adelaide.

Supervisors: Dr. Mark A. Gibson
Dr. Eric Hanssen
Prof. Mark Bartold

ACADEMIC DISSERTATION

This work does not, to the best of my knowledge, contain any material previously published or written by another person except where due reference is given in the text and has not been previously presented as a component of any other academic course. This copy of my thesis may be made available by the University of Adelaide library for loan and photocopying.

Rena M. Hirani
August 2006
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Published Scientific Presentations

Poster and Oral presentation

**2004.** Matrix Biology Society of Australia and New Zealand (MBSANZ) annual scientific meeting, Perth, Western Australia.

*Hirani, Rena M, Hanssen, Eric, Hew, Fan-Hing and Gibson, Mark*

*Binding studies of recombinant human LTBP-2 with elastic fibre components*

**Poster presentation**


*Rena Hirani, Eric Hanssen and Mark A. Gibson*

*LTBP-2 competes with LTBP-1 for interaction with fibrillin-1*

**2005.-** Matrix Biology Society of Australia and New Zealand (MBSANZ) annual scientific meeting, Victor Harbor, South Australia

*Rena Hirani, Eric Hanssen and Mark A. Gibson*

*LTBP-2 competes with LTBP-1 for interaction with fibrillin-1*

**2004.** The Australian Society for Medical Research (AMSR) annual scientific meeting, South Australian Division, Adelaide

*Rena Hirani, Eric Hanssen, Fan-Hing Hew and Mark A. Gibson*

*Binding studies of recombinant human LTBP-2 with elastic fibre components*

**2003.** The Australian Society for Medical Research (AMSR) national scientific conference, Glenelg, South Australia

*Rena Hirani, Eric Hanssen, Fan-Hing Hew and Mark A. Gibson*

*Mammalian expression of recombinant human LTBP-2 and specific binding to the N-terminal region of Fibrillin-1*

**2003.** Matrix Biology Society of Australia and New Zealand (MBSANZ) annual scientific meeting, Acheron, Victoria, Australia

*Rihani, RM, Hanssen, E and Gibson, MA*

*Mammalian expression of recombinant human LTBP-2*
Awards arising from PhD candidature

2005/06 - **Student representative** for the Matrix Biology Society of Australia and New Zealand

2005 - **Dennis Lowther award** (student poster prize 2005) awarded by the Matrix Biology Society for Australia and New Zealand in Victor Harbor, South Australia

2005 - **Travel Stipend** awarded by the Faculty of Health Sciences, the University of Adelaide to attend the Gordon Research Conference on Elastin and Elastic Fibres in New Hampshire, USA

2005 - **Research Abroad Scholarship** awarded by the University of Adelaide to attend the Gordon Research Conference on Elastin and Elastic Fibres in New Hampshire, USA

2004/05 - **Local organising committee** for the 2005 Matrix Biology Society of Australia and New Zealand (MBSANZ) South Australian meeting held in Victor Harbour

2004 - **Travel award** awarded by the Sydney Tissue Engineering and Matrix (STEAM) organisation, New South Wales, Australia to attend the Matrix Biology Society of Australia and New Zealand (MBSANZ) annual meeting in Perth
### Abbreviations

<table>
<thead>
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<th>Description</th>
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<tbody>
<tr>
<td>α-</td>
<td>alpha</td>
</tr>
<tr>
<td>β-</td>
<td>beta</td>
</tr>
<tr>
<td>Δ-</td>
<td>heat-deactivated (56°C) or (in the case of DNA constructs) has meaning “with removal of”</td>
</tr>
<tr>
<td>8-Cys</td>
<td>8-cysteine containing motif, also known as TB (TGF-β binding protein like) domain and CR (cysteine-rich) domain</td>
</tr>
<tr>
<td>BCIP-</td>
<td>5-bromo-4-chloro-3-indolylphosphate toluidine salt</td>
</tr>
<tr>
<td>βIG-H3-</td>
<td>β-inducible gene-H3</td>
</tr>
<tr>
<td>BSA-</td>
<td>bovine albumin serum</td>
</tr>
<tr>
<td>BMPs-</td>
<td>bone morphogenetic proteins</td>
</tr>
<tr>
<td>C-</td>
<td>carboxy-terminus</td>
</tr>
<tr>
<td>Ca²⁺-</td>
<td>calcium ions</td>
</tr>
<tr>
<td>CaCl₂-</td>
<td>calcium chloride</td>
</tr>
<tr>
<td>cbEGF-</td>
<td>calcium-binding epidermal growth factor-like</td>
</tr>
<tr>
<td>CCA-</td>
<td>Congenital Contractural Arachnodactyly</td>
</tr>
<tr>
<td>cDNA-</td>
<td>complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>Da-</td>
<td>Dalton</td>
</tr>
<tr>
<td>ddH₂O-</td>
<td>double distilled water</td>
</tr>
<tr>
<td>DMEM-</td>
<td>Dulbecco’s Modification of Eagles Medium</td>
</tr>
<tr>
<td>DMSO-</td>
<td>dimethyl sulphoxide</td>
</tr>
<tr>
<td>DNA-</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>E-</td>
<td>embryonic day</td>
</tr>
<tr>
<td>ECM-</td>
<td>extracellular matrix</td>
</tr>
<tr>
<td>EDTA-</td>
<td>ethylenediaminetetraacetic acid (disodium salt)</td>
</tr>
<tr>
<td>EGF-</td>
<td>epidermal growth factor</td>
</tr>
<tr>
<td>EK-</td>
<td>enterokinase enzyme</td>
</tr>
<tr>
<td>ELISA-</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>EMILIN-</td>
<td>elastin-microfibril interface located protein</td>
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<tr>
<td>FBN-</td>
<td>fibrillin</td>
</tr>
<tr>
<td>FCS-</td>
<td>foetal calf serum</td>
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<tr>
<td>GAG-</td>
<td>glycosaminoglycan</td>
</tr>
<tr>
<td>GDFs-</td>
<td>growth and differentiation factors</td>
</tr>
<tr>
<td>HCl-</td>
<td>hydrochloric acid</td>
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<tr>
<td>HEK-</td>
<td>human embryonic kidney</td>
</tr>
<tr>
<td>his₆-tag-</td>
<td>6-histidine tag</td>
</tr>
<tr>
<td>hrs-</td>
<td>hours</td>
</tr>
<tr>
<td>IPTG-</td>
<td>Isopropyl β-D-1-thiogalactopyranoside</td>
</tr>
<tr>
<td>kb-</td>
<td>kilobase</td>
</tr>
<tr>
<td>kDa-</td>
<td>kiloDalton</td>
</tr>
<tr>
<td>LAP-</td>
<td>latency-associated protein</td>
</tr>
<tr>
<td>LLC-</td>
<td>large latent complex</td>
</tr>
<tr>
<td>LTBP-</td>
<td>latent TGF-β binding protein</td>
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<tr>
<td>m-</td>
<td>mouse</td>
</tr>
<tr>
<td>mRNA-</td>
<td>messenger RNA</td>
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<tr>
<td>MAGP-</td>
<td>microfibril-associated glycoprotein</td>
</tr>
<tr>
<td>MFAP-</td>
<td>small microfibril-associated protein</td>
</tr>
<tr>
<td>MFS-</td>
<td>Marfan syndrome</td>
</tr>
<tr>
<td>MMP-</td>
<td>matrix metalloprotease</td>
</tr>
<tr>
<td>N-</td>
<td>amino-terminus</td>
</tr>
<tr>
<td>NaCl-</td>
<td>sodium chloride</td>
</tr>
<tr>
<td>NBCS-</td>
<td>new born calf serum</td>
</tr>
<tr>
<td>NBT-</td>
<td>nitro-blue tetrazolium chloride</td>
</tr>
<tr>
<td>NEAA-</td>
<td>non-essential amino acids</td>
</tr>
<tr>
<td>Ni-</td>
<td>nickel</td>
</tr>
<tr>
<td>nm-</td>
<td>nanometers</td>
</tr>
<tr>
<td>NRS-</td>
<td>normal rabbit serum</td>
</tr>
<tr>
<td>OCT-</td>
<td>optimal cutting temperature compound</td>
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</table>
OMIM- online Mendelian inheritance in man
PBS- phosphate-buffered saline
PCR- polymerase chain reaction
PO4- phosphate buffer
PVDF- polyvinylidene difluoride
r- recombinant
rb- rabbit
RGD- arginine-glycine-aspartic acid motif
RNA- ribonucleic acid
RT-PCR- reverse transcriptase-polymerase chain reaction
SCID- severe combined immune deficiency
SDS- sodium dodecylsulphate
SDS-PAGE- sodium dodecylsulphate-polyacrylamide gel electrophoresis
SLC- small latent complex
TGFβRI or II- TGF-β type I and II receptors
TB- TGF-β binding protein like domain, also known as 8-Cys (8-cysteine containing) motif and CR (cysteine-rich) domain
TBS- tris-buffered saline
TGF-β- transforming growth factor-β
TMB- tetramethylbenezidine substrate
TTX- tris/tween-20/triton X-100 buffer
U- unit(s)
UTR- untranslated region
V- volts
v/v- volume for volume
w/v- weight for volume
WMS- Weill-Marchesani Syndrome
x- times
X-Gal- 5-bromo-4-chloro-3-indolylbeta-D-galactopyranoside
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