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Expression and purification of CFM2 and Filamin A repeat 10 domain

A thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Biochemistry at Massey University, Manawatu, New Zealand.

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Abstract

Filamins are a group of proteins that interact with over 60 other proteins. Mutations to the Filamin A gene results in a spectrum of disorders including Otoplatodigital spectrum disorder type 1, Otoplatodigital spectrum disorder type 2, Frontometaphyseal dysplasia, Melnick-Needles syndrome and Periventricular Nodular Heterotopia. All cases of Melnick-Needles syndrome can be accounted for by mutations in repeat 10. Using a yeast-2 hybrid assay Professor S.P Robertson identified the protein FAM101A (the protein is alternatively named CFM2) that associated with Filamin A repeat 10. CFM2 was found to interact with itself in a yeast-2-hybrid screen, suggesting homo-dimerisation properties in addition to Filamin A repeats 10 and 21 binding affinity. If CFM2 dimerises and binds to repeat 10 and 21 it is possible that Filamin A’s function will alter, thus altering the properties of the cytoskeleton. To investigate the interaction between Filamin A repeat 10 and CFM2, each was subcloned into an *E.coli* plasmid vector fused to a purification tag. Purification of CFM2 failed due to misfolding, this upholds later work that claims CFM2 cannot fold correctly without the presence of vertebrate Filamin. Filamin A repeat 10 purification went well but the fusion was unable to be concentrated without precipitating out of solution. Also the GST purification tag could not be cleaved without secondary cleavage products forming. Pull-down of C2C12 mouse fibroblast cell lysate using the GST-Filamin A repeat 10 fusion as the probe did not identify any other proteins that bind Filamin A repeat 10.
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**Abbreviations**

AMP  ampicillin
LB   Luria broth
*E.coli*  *Escherichia coli*
MQ   milli-q
DNA  deoxyribonucleic acid
EDTA ethylenediaminetetraacetic acid
PCR  polymerase chain reaction
APS  ammonium persulfate
SDS  sodium dodecyl sulfate
DTT  dithiothreitol
DNase deoxyribonuclease
cDNA complementary deoxyribonucleic acid
TEMED *N*-tetramethylethylenediamine
Tris tris (hydroxymethyl) aminomethane
BME  2-Mercaptoethanol
PPU  Precision plus protein unstained (Bio-rad)
FLNAR10 Filamin A repeat 10
IPTG isopropyl β-D-1-thiogalactopyranoside
dNTP deoxyribonucleotide triphosphate
MSC mesenchymal stem cell
ABD  actin-binding domain
OPD1 Otoplatodigital spectrum disorder type 1
OPD2 Otoplatodigital spectrum disorder type 2
FMD  Frontometaphyseal dysplasia
MNS  Melnick-Needles syndrome
F-actin filamentous actin
G-actin globular actin
PVNH Periventricular Nodular Heterotopia
EtBr ethidium bromide
Ig  Immunoglobulin
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