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Expression and purification of CFM2 and Filamin A repeat 10 domain

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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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