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The Human Myostatin Precursor Protein: Structure, Function and Amyloid Formation.

Implications for the muscle wastage disease sporadic inclusion body myositis

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Abstract

Myostatin is a major player in the regulation of mammalian muscle growth and development, maintaining the balance between proliferation and differentiation pre-natally and the quiescence of satellite cells in adults. An absence or overexpression of myostatin results in double-muscling and cachexia respectively, placing myostatin as a promising target in the treatment of muscle wastage diseases.

As a transforming growth factor- β superfamily member, myostatin is produced as a precursor protein, consisting of a propeptide region N-terminal to the growth factor domain. Cleavage of the precursor between the domains forms the myostatin latent complex, an inhibitory structure which is exported from the cell where a second cleavage event releases the active myostatin growth factor. The precursor protein, propeptide, and latent complex play important roles in the regulation of myostatin. However, their structure and function are poorly understood, and a possible role for the myostatin precursor protein in the muscle wastage disease sporadic inclusion body myositis, suggests that pre-growth factor forms of myostatin may be additional important therapeutic targets.

This thesis presents an investigation into the structure and function of the myostatin precursor protein, the latent complex, and the propeptide region within these, with comparisons to a mutant form of myostatin responsible for the naturally-occurring double-muscling phenotype of the Piedmontese cattle breed. Results suggest that the diverse functions of the propeptide region are facilitated by regions of intrinsic disorder within a primarily structured domain, and that conformational alterations accompany the precursor to latent complex transition, resulting in a tighter inhibitory structure. Comparative analyses between the wild-type and mutant proteins suggest that the Piedmontese phenotype is due to a reduced capacity for covalent dimerisation and significant structural alterations within the type I receptor-binding domain. Investigation into misfolded myostatin precursor protein found that the precursor is able to form cytotoxic amyloid aggregates and mature fibrils under partially denaturing conditions, suggesting a possible mechanism for the role of the myostatin precursor in sporadic inclusion body myositis.

Together, these novel results contribute important information towards an understanding of myostatin structure, function and regulation in both normal and disease scenarios.

~ The answer to a question begins with the question itself ~

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A PhD is more than a degree. It is a journey of self-discovery, a rite of passage. While there is much to be gained, some things are discarded. At the end you look back and discover you are in a much better place than when you began.

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Abbreviations

A β	Amyloid beta protein
A β PP	Amyloid beta precursor protein
ACN	Acetonitrile
β ME	Beta-mercaptoethanol
BMP	Bone morphogenic protein
C313Y	Piedmontese mutation (cysteine to tyrosine at position 313)
CD	Circular dichroism
DTT	Dithiothreitol
ECM	Extracellular matrix
EDTA	Ethylenediaminetetra-acetic acid
ER	Endoplasmic reticulum
GDF	Growth and differentiation factor
GndHCl	Guanidine hydrochloride
HCA	Hydrophobic cluster analysis
IAPP	Islet amyloid polypeptide
IDP	Intrinsically disordered protein
IPTG	Isopropyl beta-D-thiogalactoside
kB	Kilobases
kDa	Kilodaltons
LB	Luria broth
LB-amp	Luria broth containing 100 μ g/mL ampicillin
MstnGF	Myostatin growth factor
MstnPP	Myostatin precursor protein
MWt	Molecular weight
NF- κ B	Nuclear factor kappa B
NMR	Nuclear magnetic resonance
NR	Non-reducing (SDS-PAGE)
PCR	Polymerase chain reaction
PrP	Prion protein
R	Reducing (SDS-PAGE)
ROS	Reactive oxygen species
RP-HPLC	Reverse phase-high performance liquid chromatography

SAXS	Small angle X-ray scattering
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
sIBM	Sporadic inclusion body myositis
TCEP	Tris 2-carboxyethyl phosphine
TEM	Transmission electron microscopy
ThT	Thioflavin T
TGF- β	Transforming growth factor beta
T _m	Melting temperature
UPR	Unfolded protein response
VEGF	Vascular endothelial growth factor
WT	Wild-type