The expression and regulation of *lama1*, a gene encoding Laminin alpha 1, during zebrafish development

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Abstract

The Hedgehog (Hh) signalling pathway is one of the major signalling pathways governing embryonic and adult development in bilateria, yet many of the direct targets of Hh signalling remain unknown. Here, I use the zebrafish embryo to investigate two potential Hh target genes that were identified in the mouse. These are *lama1*, encoding Laminin α1, and *c125*, a novel gene hypothesised to play a role in motor neuron development.

Laminins are conserved heterotrimeric glycoproteins, of which there are at least 16 different isoforms, generated from Laminin α, β, and γ subunits. Laminins are an essential component of basement membranes (BMs), which are specialised forms of the extra-cellular matrix (ECM) crucial for normal patterning, proliferation, migration, and differentiation of many different cell types. Mutations in *lama1* cause embryonic lethality in mouse and zebrafish, demonstrating that Laminin α1 function is critical for embryonic development.

The aims of this study were to assess the expression of *lama1* and *c125* during zebrafish embryonic development, and to explore a possible role for Shh in their regulation. I also aimed to determine the regulatory sequences controlling *lama1* expression using a bacterial artificial chromosome (BAC) transgenic zebrafish approach. My data reveal that the enhancer elements controlling zebrafish *lama1* expression are located within intron 1 of *lama1*. Within this intron, I have uncoupled the enhancers that regulate *lama1* expression in the muscle fibres, from enhancers that regulate *lama1* expression in the anterior CNS, neural tube, and the eye. These findings are of particular importance because ectopic expression of *lama1* in mouse models of congenital muscular dystrophy restores normal muscle function. Therefore, the characterisation of *lama1* regulation provides a framework for future studies to identify drugs stimulating endogenous *lama1* expression in dystrophic muscles.

Abbreviations

Ace	Acerebellar
ADAM	A Disintegrin and metalloproteinase
AER	Apical ectodermal ridge
AMP	Ampicillin
BAC	Bacterial artificial chromosome
bHLH	Basic helix loop helix
BM	Basement membrane
BMP	Bone morphogenetic protein
Boc	Brother of Cdo
Cdo	Cell-adhesion molecule-related, down-regulated by oncogenes
CHIP	Chromatin immunoprecipitation
Ci	Cubitus interruptus
CMD	Congenital muscular dystrophy
CNE	Conserved non-coding element
CNS	Central nervous system
Cos2	Costal 2
Dhh	Desert Hedgehog
DIG	Digoxygenin
dnPKA	Dominant negative protein kinase A
Dtr	Detour
ECM	Extra-cellular matrix
EMSA	Electrophoretic mobility shift assay
ENU	N-ethyl-N-nitrosourea
EtOH	Ethanol
FAK	Focal adhesion kinase
FGF	Fibroblast growth factor
FL	Full-length
Fu	Fused
Gas1	Growth arrest-specific 1
GFP	Green fluorescent protein
Hh	Hedgehog
HIP	Hedgehog-interacting protein
HSPG	Heparan sulphate proteoglycan
Ihh	Indian hedgehog
ILK	Integrin linked kinase
KAN	Kanamycin
LG	Laminin globular
MFF	Medial fast fibre
MMP	Metalloproteinase

MP	Muscle pioneer
MPC	Myogenic progenitor cell
MRF	Myogenic regulatory factor
MTJ	Myotendinous junction
PCR	Polymerase chain reaction
PKA	Protein kinase A
PSM	Pre-somitic mesoderm
Ptc	Patched
RA	Retinoic acid
RARE	Retinoic acid-responsive element
RT-PCR	Reverse transcription polymerase chain reaction
Shh	Sonic Hedgehog
Smo	Smoothened
Smu	Slow-muscle-omitted
SSF	Superficial slow fibre
SuFu	Suppressor of Fused
TGF	Transforming growth factor
Twhh	Tiggywinkle Hedgehog
Ubo	U-boot
UTR	Untranslated region
WISH	Whole-mount in situ hybridisation
Yot	You-too

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