

## **Abstract**

Limb tissues have a dual origin. The lateral plate mesoderm gives rise to cartilage, connective tissue and the skeletal elements of the limbs whereas cells from the dermomyotome migrate into the limb field and give rise to the limb musculature. The cells differentiate into myotubes, aggregate into a dorsal and ventral mass before undergoing sequential splitting events to give rise to individual muscles. Myogenic Regulatory Factors (MRFs) are one of the earliest markers of muscle differentiation and can be detected in the limb myocytes around embryonic stage HH 20.

MicroRNAs are 22 nucleotides long non-coding RNA molecules. They are endogenously expressed in plants and animals, where they are transcribed by RNA Polymerase III or II as a single strand called a primary miRNA (pri-miRNA) which has some internal complementarity that cause it to take on the signature stem loop structure. The pri-miRNA is cleaved by endonuclease Drosha and the resulting precursor miRNA (pre-miRNA) is exported out of the nucleus by Exportin and is then cleaved by Dicer, leaving a miRNA:miRNA\* duplex which is unwound before the miRNA binds to a RNA induced silencing complex (RISC). The miRNA can then exert post translational regulation by either binding to a target mRNA and preventing translation or by causing the mRNA to be cleaved.

The muscle specific miRNAs MiR-1, MiR-206 are known to enhance the muscle differentiation process and inhibiting the muscle cell proliferation whilst MiR-133 is known to enhance muscle cell proliferation whilst hindering differentiation. Correct temporal and special expression of these miRNAs result in the correct development of muscles.

It has been shown that MRFs can induce the ectopic expression of muscle specific miRNAs and other MRFs in the neuraltube. Progenitor cells that give rise to the limb muscles differ in several ways from cells that give rise to the axial muscles. To test if the MRFs were able to induce the expression if these miRNAs in the limbs, we first took a look at their endogenous expressions in the distal forelimb in chick embryos at embryonic stage HH35. All muscles showed ubiquitous expression of all three muscle specific miRNAs with no bias towards fast or slow muscles. Next, to examine if ectopic expression of MRFs could induce miRNA expression in the limbs, we infected developing limb buds with concentrated viral particles of RCAS-MRF. No ectopic MRF expression could be seen in the limbs despite successful RCAS infection. To investigate further we infected chick DF1 cells with the RCAS-MRF that gave us the same results. On examining the RCAS-MRF construct itself we discovered, the

construct was missing the MRF insert therefore was able to infect the cells and express endogenous RCAS genes but no MRF was being expressed.

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## **Abbreviations**

AB2	Abductor Digiti 2
AB4	Abductor Digiti 4
Ab. I	Abductor Indicis
Ab. M	Abductor Medius
AD2	Adductor Digiti 2
ADAM	A Disintegrin and Metalloprotease Domain
Ad. I	Adductor Indicis
AER	Apical Ectodermal Ridge
Anc	Anconeus
AMB	Ambiens
BBR	Boehringer Blocking Reagent
BCIP	5-Bromo-4-chloro-3-indolyl phosphate
bLHL	Basic Helix Loop Helix
BMP	Bone Morphogenetic Protein
CFC	Caudofemoralis Pars Caudalis
CFP	Caudofemoralis Pars Pelvica
cDNA	Complementary DNA
DAPI	4',6-diamidino-2-phenylindole
DEPC	Diethylpyrocarbonate
DF	Dermofibroblast
DIG	Digoxigenin
DIG-AP	Anti - Digoxigenin - Alkaline Phosphatase
DMEM	Dulbecco's Modified Eagle Medium

DNA	Deoxyribonucleic Acid
DTT	Dithiothreitol
EB4	Extensor Brevis Digiti 4
EDC	Extensor Digitorum Communis
EDL	Extensor Digitorum Longus
EHL	Extensor Hallucis Longus
EIB	Extensor Indicis Brevis
EIL	Extensor Indicis Longus
EMB	Extensor Medius Brevis
EML	Extensor Medius Longus
EMR	Extensor Metacarpi Radialis
EMU	Extensor Metacarpi Ulnaris
En-1	Engrailed-1
Ent	Entepicondyloulnaris
EP3	Extensor Proprius 3
FB	Fibularis Brevis
FBS	Fetal Bovine Serum
FCL	Flexor Cruris Lateralis
FCLA	Flexor Cruris Lateralis Pars Accessoria
FCLP	Flexor Cruris Lateralis Pars Pelvica
FCM	Flexor Cruris Medialis
FCU	Flexor Carpi Ulnaris
FDL	Flexor Digitorum Longus
FDP	Flexor Digitorum Profundus
FDQ	Flexor Digiti Quarti

FDS	Flexor Digitorum Superficialis
FGF	Fibroblast Growth Factor
FHB	Flexor Hallucis Brevis
FHL	Flexor Hallucis Longus
FI	Flexor Indicis
FITC	Fluorescein Isothiocyanate
FITC-AP	Anti - Fluorescein Isothiocyanate - Alkaline Phosphatase
FL	Fibularis Longus
FP2	Flexor Perforatus 2
FP3	Flexor Perforatus 3
FP4	Flexor Perforatus 4
FPP2	Flexor Perforans et Perforatus 2
FTI	Femorotibialis Internus
FTE	Femorotibialis Externus
FPP3	Flexor Perforans et Perforatus 3
GE	Gastronemius Externus
GI	Gastrocnemius Internus
GM	Gastrocnemius Intermedius
GS	Goat Serum
HDAC 4	Histone Deacetylase 4
HGF	Hepatocyte Growth Factor
HH	Hamburg and Hamilton stage
Hox	Homeobox
IC	Iliotibialis Cranialis
IF	Iliofibularis

IFE	Iliofemoralis Externus
IFI	Iliofemoralis Internus
IL	Iliotibialis Lateralis
IOD	Interosseus Dorsalis
IOP	Interosseus Palmaris
ISF	Ischiofemoralis
ITC	Iliotrochantericus Caudalis
ITCM	Iliotrochantericus Medius
ITCR	Iliotrochantericus Cranialis
LB	Luria Broth
Lbx-1	Ladybird homeobox -1
Lmx-1	LIM Homeobox 1
LNA	Locked Nucleic Acid
MABT	Maleic Acid , Sodium Chloride, NaCl, Tween-20
MEF 2	Myocyte Enhancer Factor 2
miR / miRNA	microRNA
MRF	Myogenic Regulatory Factor
NBT	Nitro Blue Tetrazolium
N-CAM	Neuronal Cadherin
NTM	Sodium Chloride, NaCl, Tris (HCl; pH 9.5), Magnesium Chloride, MgCl <sub>2</sub>
NTMT	Sodium Chloride, NaCl (0.1 M), Tris (HCl) pH 9.5 (0.1 M), Magnesium Chloride, MgCl <sub>2</sub> (50 mM), Tween-20 (10% v/v)
OBT	Obturatorius
P	Plantaris

Pax-3	Paired Box 3
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PFA	Paraformaldehyde
PIF	Puboischiofemoralis
PP	Pronator Radius
PS	Pronator Superficialis
Pre-miRNA	Precursor microRNA
Pri-miRNA	Primary MicroRNA Transcripts
PZ	Progress Zone
RCAS	Replication-Competent Avian Sarcoma-Leukosis Virus
RCAS-MRF	Replication Competent Avian Sarcoma-Leukosis Virus with a Myogenic Regulatory Factor Insert
r-Fng	Radical Fringe
RISC	RNA Induced Silencing Complex
RNA	Ribonucleic Acid
RNAsin	Ribonuclease Inhibitor
SOC	Super Optimal broth with Catabolite repression
SRF	Serum Response Factor
TAE	Agarose melted in Tris base, acetic acid and EDTA, Ethylenediaminetetraacetic Acid
TBST	Tris-Buffered Saline Tween-20
TC	Tibialis Cranialis
UMD	Ulnimetacarpalis Dorsalis

UMV	Ulnimetacarpalis Ventralis
V-CAM	Vascular Cadherin
ZPA	Zone of Polarizing Activity
3'-UTR	3' Untranslated Region